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Studies in Asymptomatic Primary Hyperlipidaemia

Clinical, biochemical and physiological investigations

By Anders G Olsson

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Petrus and Augusta Hedlunds Stiftelse Hans och Loo Ostermans fond,
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Hence the sages did not treat those who were already ill they instructed those who were not yet ill To administer medicines to diseases which have already developed is comparable to the behaviour of those who begin to dig a well after they have become thirsty and of those who begin to cast weapons after they have already engaged in battle.

Huang Ti (The Yellow Emperor)
(2697 2597 B C.)

The present thesis is based on the following papers, referred to by Roman numerals

I Anders G Olsson and Lars A. Carlson Studies in asymptomatic primary hyperlipidaemia. I. Types of hyperlipoproteinaemias and serum lipoprotein concentrations, compositions and interrelations. Acta med. scand. Suppl. 580. 1975 In press.

II Anders G Olsson Studies in asymptomatic primary hyperlipidaemia. II. Clinical findings. Acta med. scand. 1975 In press.

III Lars-Göran Ekelund and Anders G Olsson Studies in asymptomatic primary hyperlipidaemia. III Working capacity Acta med scand. 1975 In press.

IV Anders G Olsson, Lars-Göran Ekelund and Lars A Carlson Studies in asymptomatic primary hyperlipidaemia. IV ECG at rest and during exercise and its relation to various lipoprotein classes. Acta med. scand. 1975 In press.

V Anders G Olsson and Brita Eklund Studies in asymptomatic primary hyperlipidaemia. V Peripheral circulation. Acta med. scand. 1975 In press.

VI. L. E. Böttiger L. A. Carlson, L.-G. Ekelund and A. G. Olsson. Raised erythrocyte sedimentation rate in asymptomatic hyperlipidaemia. Brit. Med. J 2, 681 1973

Abbreviations

IHD =	ischaemic heart disease
HL	hyperlipidaemia
HLP	hyperlipoproteinaemia
LP	lipoprotein
VLDL	very low density LP
LDL	low density LP
HDL	high density LP
IT	inclination time
ESR	erythrocyte sedimentation rate

CONTENTS

INTRODUCTION

Atherosclerosis is a chronic disease of the arteries developing over many years (39). Clinical manifestations of the disease such as myocardial infarction and intermittent claudication are the late consequences of a long-lasting pathological process of the vessel wall.

Great efforts have been made during the last decades to elucidate the pathogenesis of atherosclerosis. Risk factors" for clinical manifestations of the disease e. g. ischaemic heart disease (IHD) have then been identified. The presence of such risk factors in a subject is statistically associated with an increased risk for development of IHD. Important risk factors for IHD are hyperlipidaemia (HL) (9 23 40) glucose intolerance (15,21), cigarette smoking (9 13 22, 40) and hypertension (9 21 40).

Elevated concentrations of serum cholesterol and triglycerides (TG) are known to be closely associated with atherosclerotic diseases. This knowledge is based on e. g. experiments utilizing animal models, clinical investigations (1 7 31) and epidemiological surveys (9 21 40).

The serum lipids such as cholesterol and TG are bound to protein to form lipoproteins (LPs). In the fasting state three major LPs can be isolated very low (VLDL) low (LDL) and high (HDL) density LP. These three LPs have *different* biochemical, metabolic and pathogenetic properties. For example VLDL contain mainly TG while LDL re cholesterol rich. While elevations of LDL and VLDL have been associated with a premature development f IHD (10) it has been proposed that a *reduction* serum HDL *may hasten the development of atherosclerosis (30).* Total serum cholest rol nd TG are the sums of these three different LPs. The most useful method to determine the concentrations of the LPs is preparative ultracentrifugation but unfortunately it is a complicated and costly method.

Elevation of one or more LPs is called hyperlipoproteinaemia (HLP). A typing system originally introduced by Fredrickson (16) and later modified (2) based on elevations of different LPs is currently widely used.

Such approaches as clinical studies and epidemiological surveys have shown that subjects with atherosclerotic manifestations often have HL (7 31) and that apparently healthy subjects with HL more often develop atherosclerotic disease than others (9 23). The use of these research tools thus has indicated possible pathogenetic mechanisms underlying atherosclerosis.

However in some respects these methods suffer from disadvantages. In *retrospective* clinical and epidemiological studies of e. g. subjects who have had a myocardial infarction, the atherosclerotic manifestation in itself might have influenced the LP concentrations as well as other risk factors. Also a number of subjects have died from the disease and are thus not available for examination. Clinical investigations also suffer from lack of representativity as patients in a hospital most often are highly selected. *Prospective* epidemiological studies on the other hand, require a large number of subjects for investigation, are time consuming and therefore costly. It is therefore hardly feasible to undertake elaborate examinations such as quantitative LP analysis with a prospective approach and no prospective study with quantitative LP determination and typing of HLP has yet been published. Therefore very little information is available on the role of different LPs – the physiological entities of the serum lipids – in th pathogenesis of atherosclerosis as investigated in epidemiological studies. Furthermore results from epidemiological surveys cannot elucidate pathogenetic mechanisms but only indicate possible etiological factors (risk factors"). On the other hand use could be made of the information gained in the epidemiological study. Thus, with other methods such as clinical investigations, work on animal models and in *in vitro* studies, causative relations might be established between the "risk factors"

identified in the epidemiological study and the disease.

In recent years there has been a growing int rest in the prevention of disease This has resulted in the establishment of health control centres where subjectively healthy individuals are examined for early signs and symptoms or established risk factors of various diseases In this regard great interest has been focussed on the possibility of the prevention of atherosclerotic manifestations such as IHD Although the exact role of HL in the development of atherosclerosis is not known, efforts have been mad to decrease serum lipid levels by diet or drugs in the hope of avoiding or postponing overt atherosclerotic disease (primary prevention). Encouraging although not conclusive results (12, 29) have been reported on the effect on mortality and morbidity in IHD by lowering serum lipid concentrations by diet. Also, a primary preventive trial with a serum lipid lowering drug (clofibrate) is u der way (70).

The present study should be seen against the background outlined above

Out of a basal population of about 20 000 bjects, a little more than 300 individuals in the hest 3—4 per cent of the serum cholesterol and range but otherwise healthy were examined in detail. These examinations comprised quantitative LP determination and assessment of subclinical signs of arterial disease of different location. Provided that the methods used to detect atherosclerosis are sensitive and specific a closer linking of LP abnormalities to the development of atherosclerosis could thereby be obtained, although it could not establish pathogenetic mechanisms. Primary preventive measures could then be taken.

Both epidemiological and clinical methods have been used in this new approach. Epidemiological, because we have been working with a defined basal population — health control population. Ou interest has focussed on a major risk f ctor discovered in epidemiology — HL Clinical because by selecting a small number of subjects carrying the risk factor it has been possible to perform detailed investigations such as quantitative LP analysis. Some of the usual drawba ks of both epidemiological studies and conventional clinical studies thus have been avoided A these invest gations were performed *between* the diagnosis of HL and its potential clinical consequence — overt atherosclerotic disease — we have called this approach an *interspective* study in contrast to retrospective and prospective studies.

AIM OF THE PRESENT STUDY

The aim of the present study was threefold.

First, to determine which types of HLP can be identified in asymptomatic subjects who at a health control are found to have marked HL. Furthermore to study in more detail distributions of the different LPs in HLP their composition and interrelationships and to analyze these against the background of current concepts in LP metabolism.

Second, to ascertain whether these subjects had any clinical characteristics besides HLP

Third, to determine the frequency of subclinical signs of coronary artery disease and peripheral vascular disease by non-invasive methods and to relate these findings to the LP concentrations in order to quantify the pathogenetic role of diffe rent LP abnormalities.

SUBJECTS (I)

All subjects and controls of the study came from the same source the Metropol Health Control Centre (head Sture Helander M. D) The basal population comprised about 20 000 subjects examined for their serum lipids during the course of 4 years About 700 subjects were found to have serum cholesterol and/or TG concentrations above 350 mg/100 ml and 3 5 mmol/l respectively All subjects with present or previous diagnosis or symptoms of atherosclerotic disease hypertension, secondary HL, other major illnesses such as diabetes or malignant diseases or who had any chronic drug treatment were excluded This resulted in a final sample with asymptomatic primary HL of 314 subjects. A detailed description of reasons for exclusio a d number of excluded subjects are given in paper I. All subjects of the present study were thus subjectively healthy professional people.

A control group of 1 8 subjects was obtained from the same health control who had on two occasions had serum cholesterol and TG levels below 300 ml/100 ml and 2.00 mmol/l respectively. In other respects identical criteria for participation were applied to them. The participation rate for the subjects with HLPs was 93 per cent and the controls 77 per cent.

METHODS

Serum lipid and lipoprot in determinations

Screening blood samples for serum cholesterol (4) and TG (25) determination were drawn at Metropol and analysed at King Gustaf V Research Institute.

About 3 months after HL had been diagnosed, LP analysis was made by prepar tive ultracentrifugation (6) and pape electrophoresis. Serum was inspected after standing overnight at 4 C as a qualitative test for chylomicrons. VLDL was separated from LDL and HDL in the preparative ultracentrifuge at d=1 006. The top fraction then contained VLDL. The bottom fraction was recentrifuged at d=1 063 separating LDL and HDL. The three separate VLDL, LDL and HDL fractions were extracted f r determination of cholesterol and TG concentrations. The average recovery varied between 93 and 99 per cent in the diff rent types of HLP

LP paper electrophoresis (26) was performed on whole serum nd on top a d bottom fractions after separation in the pr parative ultracentrifuge at d=1 006 This was done in order to detect the presence of "floating β" LP

Typing of h pe lipoproteinaemia

Typing of the HLP serum was performed according to principles of Fredrickson et al. (16) as modified n a WHO memorandum (2). The criteria used are described in Figure 1 Cut off points used for d fining elevated concentrations were 220 mg/100 ml for LDL cholesterol and 1 25 mmol/l for VLDL TG. These values were rounded off means +2SD of a f rmerly run normal male material in Stockholm and thus arbitrarily chosen. Type III and V were characterized by VLDL TG concentration above 1 25 mmol/l *and* floating β in the former and fasting chylomicronaemia i the latter respecti ly

Physiological methods (III IV V)

To detect xercise ST segment depressions nd to determine wo king capacity work test was pe formed on a heart rate controlled bicycle ergometer to near maximal heart rat (14). The subjects worked for six-minute periods on consecuti e loads beginning with a heart rate of 90 and increasing every sixth minute by 20 beats/min. With this method a direct instead of an intra- or extrapolat d m asure was achieved fo the work performed t the heart rates of 130 150 a d 170 beats/min. The work performed t given heart rate was used as an expression for working capacity ECG was interpreted ccording to the Minnesota code (33) The interpreter did not know whether the ECG was recorded from a subject with HLP or a control subject. Details are given in paper III.

To detect subclinical signs of peripheral vascular disease digital pulse plethysmography of the lower limb was performed (28) This method have proved to be particularly sensitive in the detection of vascular abnormality in early stages of peripher al atherosclerosis (42) Details of the m thod is given in paper V

Other methods

Intravenous glucose tolerance test ESR and non-lipid chemical methods were performed according to the clinical routine of the hospital.

The statistical treatment of parametric data was performed according to S edecor (36). For comparisons of non-parametric data, Fisher s exact probability test was used (34). Linear and logistic regressi n analysis was perf rmed according to the computer program BMD07R.

RESULTS AND COMMENTS

1 LIPOPROTFINS (1)

The different steps involved in the achievement of the final interspective sample of subjects with asymptomatic primary HLP are described in paper I. LP data are also detailed in this paper

Types of hyperlipoproteinaemias

Five different types of HLP (Fig. 1) were diagnosed in both men and women. In addition about 20 per cent of the 314 subject who had HL at screening had "normal" LP pattern at LP analysis three months later. This group was called N In men type IV HLP was most frequent followed in order of frequency by N II A, II B III and V HLP In women type II A was most abundant followed by N IV II B III and V HLP Type I HLP was not seen and is so far not reported from Sweden.

The effect of changing the cut off points in fining elevated concentrations of VLDL TG and DL cholesterol on allocation in the various types HLP was studied.

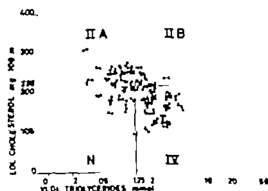


Fig 1 VLDL TG and LDL cholesterol concentrations in males (open symbols) and females (closed symbols) in the interspective sample. The horizontal and vertical lines indicate the cut off points in the typing f hyperlipoproteinaemia. On this basis the sera ere allocated t type II A II B, IV and N as given in the Figure. For types III (△and▲) and V additional criteria are needed for typing (see te t) VLDL TG re given in logaritmic scale.

As expected the higher the cut off point the more sera were classified as N However the most dramatic effect of going from low to high cut off points was in both sexes a pronounced reduction of the number of type II B Thus, changes in the cut off points not only changed the number of HLP subjects but also the relative frequencies of different types of HLP

Non-diagnostic lipoprotein abnormalities.

Apart from the abnormalities inherited in definition other LP abnormalities were present in some of the types of HLP

1 Type III HLP had high VLDL cholesterol concentration in relation to VLDL TG concentration.

2. Types characterized by VLDL TG elevation (II B III IV and V) had *relatively* low levels of LDL cholesterol and HDL cholesterol and high levels of LDL TG and HDL TG

In order further to analyze the compositions and interrelations of the LP classes all HLP subjects were considered as one population, and correlation and regression analysis was performed on the whole sample of HLP subjects.

Lipoprotein lipid composition

Within each LP fraction highly significant correlations between TG and cholesterol were found in VLDL indicating that this LP had a constant composition in this regard. For LDL and HDL no or only weak correlations existed between cholesterol and TG thus indicating *heterogeneity* of these two LPs.

Lipoprotei interrelationships

Both LDL and HDL showed fairly strong rel tions to VLDL. Therefore VLDL TG were used as independent ariable in correlation analysis. LDL cholesterol was significantly negatively related to VLDL TG. Thus, in type V on the one hand in which VLDL TG is usually very high LDL cholesterol was i all cases below 100 mg/100 ml (Fig. 1) The main implication of this association is that only low and never high LDL cholesterol concen-

trations were seen with high VLDL TG levels. Type II A subjects with extremly elevated LDL cholesterol levels, on the other hand, had low concentrations of VLDL TG (Fig. 1). Negative correlations also existed between VLDL TG and HDL cholesterol. Positive correlations with VLDL TG were found for HDL TG and for LDL TG in females.

Comment

From Figure 1 it is evident that the cut off points used in the classification of HLP into different types are arbitrary and seem artifical. A smooth and continuous negative relationship existed between VLDL TG and LDL cholesterol as the *maximal* LDL cholesterol concentration decreased with increasing VLDL TG concentrations. The divison of this continuity into different types of HLP may therefore be misleading as one easily becomes inclined to look at different types of HLP as different disease entities.

It is important to bear in mind this negative relationship between VLDL TG and LDL cholesterol in the further discussion of the relation between LP concentrations and subclinical signs of atherosclerosis.

2. FINDINGS NOT ATTRIBUTED TO ATHEROSCLEROSIS (II)

Anthropometric data

HLP women were about five years older than men. This was due to higher mean age among women at Metropol but probably also to other selective mechanisms. No difference in mean age was seen between HLP groups and controls except in women with type II A, who were older. Subjects with type IV HLP were heavier than controls. There was a tendency towards shorter body height in subjects with type II HLP. Weight/height index was higher in groups with VLDL TG elevation.

Stigmata of hyperlipoproteinaemia

(Table I). The most frequent HLP stigma was arcus corneae. This was seen to a considerable extent also in the controls. A highly significant correlation was found between the frequency of arcus and the mean LDL cholesterol concentration in the different groups.

Xanthelasmata and tendon xanthomata were mostly seen in type II A and II B HLP. Multiple tendon xanthomata were seen exclusively in type II A HLP. These subjects had higher LDL cholesterol concentrations, and the males were younger than the other type II A subjects.

Palmar xanthomata were seen only in the presence of "floating β" LP abnormality characteristic for type III HLP.

One case with eruptive xanthomata had type V HLP.

It was concluded that HLP stigmata found in a health control population could be divided into *general* (arcus corneae, xanthelasmata) which were found both in various HLPs and in controls, and *diagnostic* characterizing a defined LP abnormality (multiple tendon xanthomata = type II A HLP, palmar xanthomata = type III HLP and eruptive xanthomata = type V HLP).

Table I. Frequency of stigmata of HLP in subjects with types II A, II B, III, IV and V HLP. C = control group. N = normal LP type despite hyperlipidaemia at screening. (see text)

Group		Arcus corneae %	Xanthelasmata %	Tendon xanthomata %	Palmar xanthomata %	Eruptive xanthomata %
C	128	29	0	0	0	0
N	66	50*	6	8	0	0
II A	77	69*	10*	45	1	0
II B	76	54	19*	23	0	0
III	17	29	6	0	12	0
IV	124	38	2	2	0	0
V	4	25	0	0	0	25

RESULTS AND COMMENTS

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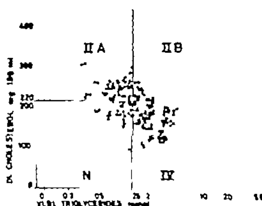


Fig 1 VLDL TG and LDL cholesterol concentrations in males (open symbols) and females (closed symbols) in the interspective sample The horizontal and vertical lines indicate the cut off points in the typing f hyperlipoproteinaemia On this basis the sera were allocated to type II A II B, IV and N, as given in the Figure I or types III (Δ and ▲) and V (and) additional criteria are needed for typing (see text) VLDL TG are given in logaritmic scale.

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frequency of ST depressions 4 1–4 4 according to the Minnesota code (30) among controls in the age groups 35–50 and above 50 years was (males/females) 8/23 and 26/44 per cent respectively It increased with age in both HLP and in controls. It was higher in females than in males. In younger subjects the prevalence of ST depressions was significantly higher than in controls in type II A and IV males and group N females. In ages above 50 significantly higher frequencies in controls were seen in males with type II B and III and females with type II A and II B HLP When all ages we e taken together significantly higher frequencies were found in all male HLP groups except N and female N II A and II B groups. The frequency of the more pronounced ST depressions (Minnesota code 4 1–4 2) was distributed between HLP groups approximately as the prevalence of 4 1–4 4 i. e. it was most abundant in type II A and II B There were no differences in frequency of ST depressions between subjects having "low and "high" blood pressure and low and high glucose tolerance. Smokers had a tendency to lower frequencies of ST depressions than non-smok rs.

From Figure 1 it was evident that the concentrations of VLDL and LDL varies continuously and that the various types of HLP are arbitrarily defined The relation between LP concentrations and ST depressions was therefore studied further by multiple linear regression analysis using age and LP concentrations of all subjects except N but including controls as independent variables. In addition smoking, diastolic blood pressure, weight/height index, working capacity ESR and k-val e at iv glucose tolerance were entered into the equation.

Age gave invariably the highest correlation to ST depressions.

When different LPs were used singly in additi to age significantly higher correlations were achieved with log VLDL TG LDL TG and LDL cholesterol in both sexes and log VLDL cholesterol in females. Furthermore th highest correlation coefficients between ST depressions and both log VLDL TG and LDL cholest rol when used singly in combination with age were achieved with parabolic functions of the LP This implied that with singly increasing LDL cholesterol or VLDL TG concentrati ns the relative frequency of ST depressions increased at low and moderate LP concentrations. But above a certain LP level the relative frequency decreased. The reason for this relation could be explained if we look at Figure 1 The higher o e of these LP is the lower the *other* If both LDL cholesterol and VLDL TG were of importance in the development of ST depressions, therefore a parabolic relationship could be ex pected when using only one LP in the explanation of ST depressions.

This was confirmed when *both* LDL cholesterol and VLDL TG were used as independent variables in the same equation as *both* LPs significantly increased the correlation coefficient in males

As no other LP but VLDL TG significantly increased the probability for ST depressions when added to LDL cholesterol the equation including the three significant terms age LDL cholesterol and VLDL TG was used to predict the occurrence of ST depressions. This was illustrated by tables giving *probabilities* of ST depressions with differ ent concentrations of LDL cholesterol and VLDL TG Also as an example some isoprobables are given in Figure 4 and 5

In recent years the logistic regression has been used in the analysis of epidemiologic data (18) The logistic function is very useful method in so far that the probability given for an outcome (y) is always between 0 and 1 Therefore multiple logis-

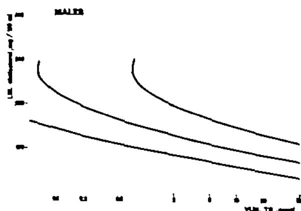


Fig 4 Probabilities (p) ("Isoprobables p 0.1 0.3 and 0.5) for ST segment depressions during exercise deter mined by LDL cholesterol and VLDL TG concentrations in men aged 50 given by the equation p – 1.16 0.014 age years 0.084 ⁴log VLDL TG mmol/l 0 0071 LDL cholesterol, mg/100 ml – 0 000013 [LDL cholesterol]²



Fig. 5 Probabilities (p) ("Isoprobables" p = 0.1 0.3 and 0.5) for ST segment depressions during exercise deter mined by LDL cholesterol and VLDL TG concentrations in females aged 50 given by the equation p – 0.84 + 0.008 age, years + 0.071 elog VLDL TG, mmol/l 0.079 x LDL cholesterol – 0.000016 [LDL cholesterol]^2

tic regression analysis was also performed in the present study using age and the most important LPs as independent variables. The resulting t values nd multiple correlation coefficients are given in II The multiple logistic regression explained t the same proportion of the total variance as ordinary multiple regression.

The square correlation coefficient (r^2) indicates the proportion of the total variance of the ST depressions that is "explained" by the different terms in the equation. Taking into account all major risk factors – age LPs smoking, blood pressure etc – only resulted in an explanation" of about 25 per cent when calculated on multiple linear and on logistic regression This fact might indicate that the statistical modul used was wrong or the existence of unrecognized pathogenetic factors in the development of ST depressions and IHD As the subjects in the present study were selected on account of HL it seems natural in case of the latter possibility to look for new pathogenetic factors for IHD within the field of lipid metabolism.

Table II T-values and square correlation coefficients (r^2) f the dependence of ST depressions on age and lipoproteins in multiple linear and logistic regression analysis

	MALES 166		FEMALES 135	
	Linear t	Logistic t	Linear t	Logistic t
Age	4 17	3 62	1 77	1 58
elog VLDL TG	2 45	2 46	1 35	1.28
LDL cholesterol	3 14	2.53	2 40	1 98
(LDL cholesterol)^2	2 24	1 78	2 19	1 74
			r^2	2
	0 23	0 25	0 14	0 14

Comment

The frequency of ST depressions during and after exercise in the control subjects of the present study is in agreement with that of normal subjects in other reports (e. g. 37) In paper IV the significance of *ST depressions (Minnesota code 4 1–4.3)* was discussed It was concluded that among subjects free of other heart disorders such as cardiomyopathy and status post myocarditis and other conditions such as anemia electrolyte disturbances or treatment with cardiotropic drugs (selection criteria in the present study) ST segment depressions of the type and magnitude reported could be considered as a probable indicator of myocardial ischaemia most often due to coronary artery disease This holds true also for women (27). The presence of ST depressions during exercise does not definitely indicate a grave prognosis in individual subjects. However the presence of ST depressions of this magnitude during and after near maximal exercise implies *substantially increased risk* of developing overt IHD (e g. 3 24)

Physical working capacity (III)

In men the physical working capacity was lower in all HLP groups than in controls, most pronounced in the younger age groups. Women with type II A had lower working capacity than controls. After correction for variation in body weight and age there remained a lower working capacity in males and females with type II A and in males with type IV HLP

The lower physical working capacity in *HLP* subjects could be due to a difference in stroke vol me. This could in turn be explained by either a lower degree of physical training or a less

effective myocardial function in HLP subjects.

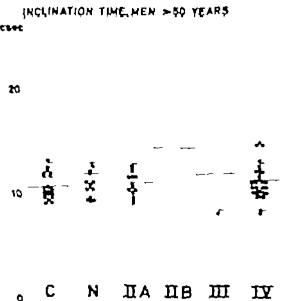
Older type II B males had a higher respiratory rate at heart rate 130 beats/min than controls despite a mean work load that should have demanded a 17 per cent lower oxygen intake. Dynamic spirometry showed a lower vital capacity in HLP subjects compared to controls, after correction for variation in age body weight and height. No differences were found in forced expiratory volume (FEV_1) As a decrease in vital capacity might be a sign of latent myocardial failure (17) and should not be a result of any difference in physical activity this finding suggests that one reason for the decreased physical fitness might be a decreased left ventricular function, possibly indicating a latent ventricular insufficiency A third factor may be a common genetic mechanism responsible for both the hyperlipoproteinaemia and the changes in physiological variables, e g vital capacity and working capacity

The dependence of the physical working capacity on various LPs according the typing system, was further studied for reasons mentioned above When corrections for variations in age and body dimensions were made the only significant LP determinants of working capacity were VLDL TG (negatively) in males ($p<0.001$) and HDL cholesterol (positively) in f males ($p<0.05$) This is in contrast to the dependence of ST depression on LPs where LDL cholesterol always emerged as the most significant LP determinant.

Peripheral circulation (V)

Inclination time (IT) has been shown to be significantly increased already when arteriography shows only slight diffuse atherosclerotic changes (42) Therefore IT is considered to be a sensitive variable in detecting early subclinical arterial disease

The IT (Fig. 6) of the lower limb was substantially prolonged in 12 per cent of the male and 7 per cent of the female HLP subjects, indicating the presence of peripheral vessel wall disease The majority of these subjects presented elevation of both cholesterol and TG A disordered TG metabolism was the most constant LP abnormality in subjects with IT prolongation not only because of high frequency of VLDL TG elevation but also



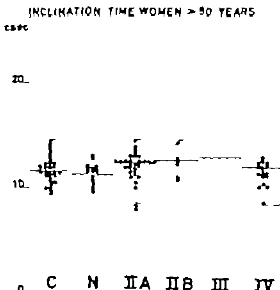


Fig 6 Inclination time in the digital pulse plethysmogram of the lower limb in males (left) and females (right) above age 50 with different types of hyperlipoproteinaemia and controls Closed symbols smokers, open symbols non-smokers. The full horizontal line indicates the mean value of the group. The dashed lines indicate the mean value ± 2SD of the control groups, C N see legend to Figure 2.

because of high LDL TG levels. Increase in LP cholesterol was often due to elevated VLDL cholesterol levels. IT prolongation was most frequently seen in connection with moderate elevations of both TG and cholesterol carried in VLDL, LDL or intermediary LP as judged from the relatively high frequency in type III HLP. On the other hand prolonged IT was not seen in connection with extreme elevations of either LDL cholesterol or VLDL TG and low concentrations of the other (Fig. 1). Ninety-five per cent of the males and 75 per cent of the females with IT prolongation were smokers. All but one of the subjects with HLP and prolonged IT had at least two "risk factors". Most subjects were above 50 years of age.

The frequency of signs of coronary artery disease as estimated by the frequency of ST depressions during exercise and of vascular disease in the lower limb as estimated by the inclination time could be compared to obtain a picture of the location of early atherosclerosis in different types of HLP. In men above 50 years the following percentage frequencies existed for ST depressions (Minnesota code 4.1—4.3)/IT prolongation: II A 53/7, II B 75/38, III 50/33 and IV 38/21. This shows that LDL cholesterol elevation seems to promote predominantly coronary artery disease and not peripheral vascular disease as the difference was very pronounced in type II A between the occurrence of ST depression and IT prolongation. When VLDL was elevated with or without LDL cholesterol elevation, the frequencies of ST depression and IT prolongation became more similar. This might suggest that the atherogenic potency of LDL cholesterol elevation is primarily confined to the coronary arteries while elevation of VLDL TG might affect both coronary and peripheral arteries.

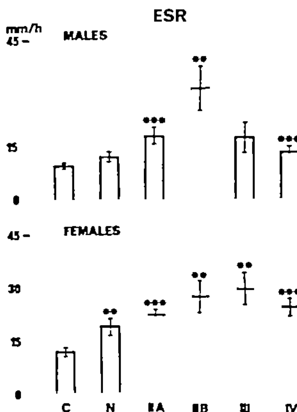


Fig. 7. Mean ESR (±SEM) in males and females with different types of HLP and in controls (C). and indicate significant difference against control groups on the 1 and 1 per cent level respectively.

ESR (II, VI)

In paper VI it was demonstrated that the ESR was significantly increased in type II A, II B and IV HLP. This paper was prepared when approximately half of the interspecti e sample was collected. The mean ESR values of the complete study are given in paper II and in Figure 7. They confirm previous observations. In addition ESR was found to be elevated in type III HLP and N groups in women.

As lysolecithin by its surface active properties might influence the ESR – a low concentration resulting in an increased ESR – the concentration of lysolecithin was determined in HLP subsamples with "very high" and "very low" ESR. There were no difference in concentration of lysolecithin between these groups.

The hypothesis was put forward that the raised ESR found in HLP subjects is caused more by hidden vascular disease than directly by serum LP levels. This was based on the fact that high ESR was not confined to any specific LP abnormality but was found in all types of HLP. Subjects with high ESR had however considerably increased frequency of ST depressions during exercise. This indirect relation between HLP and ESR may in part be explained as follows: HLP causes vascular disease (atheroscle-

roids) which may result in raised ESR. Such a relation may also explain why raised ESR has been found to be a risk f ctor for IHD (5)

The relation between HLP and ESR was studied further by multiple regression analysis including age LPs and body weight as independent variables. In females independent determinants of ESR were VLDL TG LDL cholesterol and body weight in males age and LDL cholesterol For LDL cholesterol the relation was parabolic with increasing ESR with increasing LDL cholest rol at relatively low LDL cholesterol concentrations, but decreasing ESR at high LDL cholesterol levels. Thus the dependence of ESR on different LP showed a similar pattern as that of ST depressions. This is in line with the suggestion that the high ESR in HLP is caused by hidden vascular disease

The frequency of ST d pressions during exercise was also studied in relation to ESR by regression analysis. In women ESR was a significant determinant of the probability of ST depressions when used togethe with age only Together with the LPs howeve ESR did not significantly alter the probability of ST depression

These findings confirm the earlier conclusions that the high ESR found in HLP probably is related to th degree of vessel wall disease caused by the HLP

GENERAL DISCUSSION

The basal population

The basal population was exclusively subjects attending a health control centre and subjects participating in the interspective study thus represented individuals available for active preventive medicine n the Stockholm metropolitan area As the sample was not randomly selected from the inhabit nts i Stockholm conclusions as to prevalences of HLP and of preclinical atherosclerot c disease in the Stockholm population can therefore not be drawn.

A major advantage with the interspecti e st dy was that starti g from a large sample which was screened by simpl methods, a final sample of HLP subjects which could be studied in great d tail with advanced biochemical and clinical-physiological methods was sifted out.

Classification of hyperl poproteinaemia

The advantages and disadvantages of the present system of classification of HLP were discussed (I) This classification system has major advantages in the sense that it stimulates thinking in terms of LPs and that it facilitates communication.

Disadvantages previously pointed out are that subjects often oscillate between several types within a relatively short time Also ne may easily become inclined to look erroneously at the types of HLP as separate disease ent ties of which the expression type "II B disease" is an example It has to be kept in mind that it is the plasma and not the patient, which is typed (4-P problem, ref 8). However the typing system has nothing to do with etiology or pathogenesis.

In the present study some other drawbacks were evident.

1 The cut off points must be a bitrarily defined.

2. The allocation of subjects into diff ent types of HLP was highly dependent on the cut off points chosen not only in the sense that changing the cut off points altered th number of subjects having HLP but also that the relative frequencies of the various types changed

3 Several continuous relations were inter rupted for example

a the negative relation between LDL cholesterol and VLDL TG

b for types with elevated LDL cholesterol the continuous distribution of VLDL TG

c. for types with elevated VLDL TG th continuous distribution of LDL cholesterol

However the lack of alternatives at the present moment makes the classification for HLP still a useful system.

Lipoprotein compositions and interrelationships in hyperlipoproteinaemia

The lipid composition of the different LP classes was studied by correlation analysis of all HLP subjects. No major sex differences existed.

1 In VLDL cholesterol and TG were strongly correlated compatible with a homogeneous composition of this LP class.

2 In LDL cholesterol and TG were weakly correlated in males but not at all in females. This indicates a *heterogeneity* of this LP class However several types had a characteristic pattern for this relation which was discussed against the concept that LDL is composed of at least two subclasses the TG rich LDL_1 and the cholesterol rich LDL_2. *The cholesterol content increased in relation to the TG content of LDL in the following order* Type V III II B and II A For type IV *there was a wide range of this relation* These findings suggested that there was a gradual increase in the ratio of LDL_2/LDL_1 from type V via III and II B to II A The highest ratio was seen in II A with multiple tendinous xanthomata while the type IV was very variable in this ratio

3 Cholesterol and TG were not at all related in HDL.

Possible pathogenetic mechanisms in hyperlipoproteinaemia

By analysing the composition and interrelationships of the different LPs possible underlying *metabolic abnormalities in the pathogenesis of* HLP were discussed (1)

One major function of the serum LP is to transport TG (32) The TG are synthesized in the liver and secreted into the circulation as VLDL The TG rich VLDL are then attacked by the enzyme system lipoprotein lipase which splits off fatty acids from the TG By this process VLDL lose more TG and eventually become degraded to LDL_2 This can be depicted schematically as *follows*

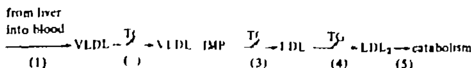
Within the course of this LP degradation abnormalities exist which are expressed as different types of HLP The concentrations of VLDL and LDL and the cholesterol/TG relation in both VLDL and LDL help to explain these abnormalities The following explanations for the different types of HLP are the most simple for each type and assume first order kinetics, (numbers referring to the various steps above ↑= increase and ↓= decrease of the steps, for detailed outline see 1)

Type II A (5)↓
Type II B (1)↑
Type III (3)↓and (4)↓
Type IV (2)↓or (1)↑depending on LDL concentrations and composition Type IV seemed to be the most *heterogeneous type* from the pathogenetic viewpoint
Type V (2)↓ and additional criteria on VLDL synthesis and/or on VLDL composition.

More complex explanations such as e g. a reduction of steps 2 4 and 5 to explain type II B and other combinations of the above mechanisms are of course also possible and probable

The role of different lipoproteins in the development of ST depressions

There are two main aspects of the clinical and physiological results of th present interspective study The *first* is that it gi es a picture of subclinical signs of disease in a sample of HLP subjects from a health contr l centr The increasing interest of the community in he lth control includ ng advanced laboratory investigations and prevention of disease makes it important to evaluate the possible clinical relevance of pathologic findings such as hyperlipidaemia With regard to HLP *it is known that apparently healthy subjects with*



IMP Intermediary particles probably simila (identical?) t th "floating β" LP of type III HLP

HLP have an increased risk for development of IHD (8). Little is known, however, of the ext nt of atherosclerosis in early asymptomatic stages of various LPs.

The present study shows that among asymptomatic subjects offered health control by their employers a number of subjects had marked HL. In these subjects there was a higher proportion of pathological findings indicating the presence of vessel wall disease both in the coronary and peripheral arteries than in subjects with normal serum lipid lev ls. These results focus the interest on the *preclinical* stage of atherosclerosis and stress the need for early detection of HL and for *primary* prevention i. e. before the onset of overt disease. As consequence of the results e peri ments are needed to try to reduce early signs of atherosclerosis in subjects with HLP by decreasing their serum LP concentrations.

The *second* main aspect of the results is the possibility to evaluate in d tail with statistical methods the role of various factors in the preclinical development of ischaemic vascular disease. In the interspective study the main emphasis has been given not only to various types of HLP in this regard but also to the quantitative ole of the various LPs.

In paper IV and V of the present study it was shown that probable signs of preclinical atherosclerosis were seen more often in subjects with HLP than in subjects with serum lipids in the normal range. That probabl preclinical signs of thero-sclerosis such as ST depressions during exercise *de facto* were found more ften in asymptomatic subjects with HLP tha in controls off red a means f investigating the importance of HLP in the early development of atherosclerosis. B t furthermore as the occurrence f ST depressions was found in all types of HLP and also in initially HL subjects with normal type of HLP w had to study *t differe t types of HLP* but *concentrations of different LPs* in order to understand the importance of HLP in the development of coronary therosclerosis.

Since as discussed bove the current typing system for HLP breaks the continuous relation of VLDL and LDL at arbitrary levels, it seemed unbiological and artificial. Instead we made use of the *actual conce trations* f the LP classes for each subject by means of multiple regression analysis to elucidate the possible atherogenic role of the different LPs. In addition other factors such as age blood pressure smoking, k-value at glucose tolerance were entered into the regression equation.

The regression analysis was used for two purposes.

First, it was used in an *analytical* sense to dete mine the importance of different LP and other factors in the development of ST depressions. It was th reby found that age invariably was most significantly correlated with ST depressions during exercise. Of LPs singly added to age LDL cholesterol wer most significant followed by LDL TG VLDL TC and VLDL cholesterol (females). HDL cholesterol was negatively but not significantly related to ST depressions. Upon age singly added non-lipid variables only ESR in women was significant. If LPs and other factors were added to age + LDL cholesterol only VLDL TG was significantly related to ST d pressions in men. It was concluded that *both* LDL cholest rol *and* VLDL TG we e of importance in the development f ST depressions during exercise.

Secondly the regression analysis was used in a *synthetic* way in order to predict the probability of having ST depressions based on significant det rminants. Age, LDL cholesterol and VLDL TG were thus used as independent terms in the equa tion. Thereby tables were constructed giving probabilities of ST depression when LDL cholesterol and VLDL TG were known (age 50). Also "isoprobables" were constructed (Fig. 4 and 5).

Implications of the combined effect of LDL and VLDL in the development of atherosclerosis

The present study has demonstrated that elevated concentrations of both LDL cholesterol and VLDL TG are sign ficant determinants of the probability of ST depressions during exercise. Provided that the ST depression of the magnitude studied reflects coronary atherosclerosis – and a considerable body f evidence indicates that this is the case – this finding has several implications.

Pathoge etic implications. Various explanations have been proposed for the ccumulation of lipids in the rterial wall (19). Many of them include a high concentration of LDL cholesterol as a neces-

sary condition in the development of atherosclerosis. The VLDL particles are however in themselves too large to penetrate the arterial intima (250–750 Å) (35) Recently Zilversmit (43) proposed a mechanism by which VLDL and chylomicrons might be ath rogenic. This mechanism takes into consideration the kinetic scheme given above (p 18). Through the action of lipoprotein lipase in the arterial wall, TG rich large VLDL molecules are converted into smaller cholesterol rich LDL particles. In th blood-artery interface cholesterol rich LDL may th refore greatly exceed the concentrations in circulating blood, especially when VLDL is increased. This theory is in accordance with the present study which showed that 1 the highest frequencies of ST depressions were found in type II B HLP and 2 that both LDL and VLDL were "independent predictors" of ST depressions.

Prognostic implications No prospective study has been published in which concentrations of LP have been determined and types of HLP assessed. However in the Stockholm Prospective Study serum TG concentrations were determined (9) In this study the highest rate of new events of IHD ... in the group which on the basis of total lipid levels was considered to ha e type II B and IV HLP This is in agreement with th result of the present study

Therapeutic implications. There are *two* pieces of evidence from prospective studies indicating the increased risk for IHD in subjects with HLP

1 Prospective studies taking serum lipids into account (9)

2 Prospective studies taking ST depression during exercise into account (24)

This calls for a more active preventive attitude particularly towards subjects e hibiting *both* these f ndings. It therefore seems nat ral to try reduce the serum cholesterol and TG levels n the hope of avoiding or postponing overt disease

In recent years a number of studies has been published dealing with th ffect of reducing serum lipids on morbidity nd mortality in IHD (e g. 12 20 9) Both diet d drugs ha e been used

Only on aspect should m this conte t be brought up to discussion W (11) nd others (38 41) have earlier reported on the effect of dietary and drug treatment on LDL cholesterol concentrations in type IV HLP Concomitantly with the reduction in VLDL TG a relation between initial LDL cholesterol and the effect on LDL cholesterol was achieved predicting that when pretreatment levels of LDL cholesterol in HLP is below about 140 mg/100 ml the LDL cholesterol will rise (11)

Assume that we have a 50 year old male patient with a type IV HLP a VLDL TG concentration of 5 mmol/l and a LDL cholesterol concentration of little more than 100 mg/100 ml a very likely combination (Fig. 1) From the "isoprobables in Fig. 8 it can be seen that this subject will have a probability of exercise ST depressions of 30 per cent We want to treat this subject with a serum lipid lowering regime (diet or drug) With this regime we assume a reduction in VLDL TG of 3 mmol/l to mmol/l a substantial decrease This decrease would according to the equation (legend to Figure 5) imply a lowering of the risk for ST depressions to about 20 per cent if the LDL cholesterol was unchanged. However according to our experience (11) the effect on the LDL cholesterol concentration by serum lipid lowering regimes such as diet or clofibrate is directly related to the pretreatment LDL cholesterol levels implying

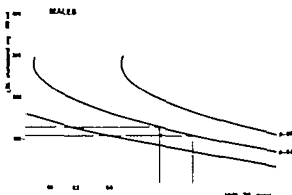


Fig 8 Effect f the reciprocal changes in VLDL TG and LDL cholesterol concentrations fter treatment with serum lipid lowering regime on the probability of ST depressions during exercise. As an example 50 years old male with an initial VLDL TG concentration of 5 mmol/l and LDL cholesterol concentration just above 100 mg/100 ml as chosen. Treatment *lowered* VLDL TG t mmol/l but *increased* LDL cholesterol to 125 mg/100 ml Thu no decrease in the probability f ST depressions as achieved (see te t)

a decrease of LDL cholesterol levels above 140 mg/100 ml and an *increase* below that level. In this case we would see an *increase* of about 20 mg/100 ml. This ends with exactly the same probability of ST depressions. It could also be said that we have given the patient a more deleterious cholesterol. According to the normal composition of VLDL with 20 per cent cholesterol, VLDL cholesterol was reduced by 60 mg/100 ml while the increase of 20 mg/100 ml in LDL cholesterol gave the same probability to ST depressions.

The differential effect on lipoprotein levels of serum lipid lowering regimes has so far not been considered in the preventive programs. Obviously this must be taken into account in the evaluation of the effects of longterm hypolipidaemic regimes on morbidity and mortality in cardiovascular disease in subjects with type IV HLP. To circumvent this effect more efficient drugs are needed.

GENERAL SUMMARY

1 To determine lipoprotein (LP) abnormalities, clinical characteristics and preclinical signs of atherosclerosis in asymptomatic subjects with hyperlipidaemia serum cholesterol and triglyceride (TG) concentrations were determined in [illegible]0 000 subjects attending a health control centre linked to their employment.

2 Three hundred and fourteen asymptomatic subjects with serum cholesterol ≥350 mg/100 ml and/or TG ≥3.50 mmol/l in the screening test but without signs or symptoms of secondary hyperlipidaemia or history of cardiovascular disease were examined further

3 LP analyses with preparative ultracentrifugation separating very low (VLDL) low (LDL) and high (HDL) density LP classes and the determination of cholesterol and TG concentrations in each fraction was performed. LP paper electrophoresis was run on whole serum and on top and bottom fractions after separation in the ultracentrifuge at *d*=1 006 Typing of hyperlipoproteinaemia (HLP) was performed according to WHO based upon the values for VLDL and LDL.

Exercise ECG was performed on [illegible] heart rate controlled bicycle ergometer The subjects worked at constant predetermined heart rates. ECG at rest and during exercise was interpreted without knowledge of whether or not the subject had HLP and coded according to the Minnesota criteria

Digital pulse plethysmography was performed on the lower limbs

Identical investigations were performed on a control group with non-elevated serum lipids.

4 The following proportions of types of HLP were found (males/females [illegible]) II A 16/37 II B 7/10 III 6/5 IV 50/4[illegible] V [illegible]1 and normal type 19/[illegible]5 Considerable differences in the number of subjects allocated into different types of HLP were noted especially for type II B HLP when moderate changes were made in the cut off points for defining elevated VLDL TG and LDL cholesterol concentrations. This demonstrates the arbitrary nature of the typing system.

5 The following non-diagnostic LP abnormalities were found VLDL cholesterol was high in type III HLP LDL and HDL cholesterol were relatively low in types of HLP characterized by VLDL TG elevation. In these types LDL TG and HDL TG were high.

6 A close relation existed between VLDL cholesterol and TG in HLP compatible with a homogeneity of this LP class. LDL and HDL were more heterogenous as regards lipid composition Type III HLP had relatively high VLDL cholesterol concentrations.

7 Several highly significant relations existed between the various LP classes. This was studied by relating different LPs to VLDL TG which had the highest correlations to other LPs A negative relation was found between LDL cholesterol and VLDL TG. Thus LDL cholesterol was never high when VLDL TG was markedly elevated. Negative correlation also existed between VLDL TG and HDL cholesterol while positive correlations were seen between VLDL TG and HDL TG and between VLDL TG and LDL TG in females.

8 The problem of classification of HLP was discussed. The present typing system of HLP is simple and convenient Disadvantages were

a. Arbitrary cut off points.

b. Small variations of the cut off points will change not only the absolute number of HLP but the relative frequencies of the types.

c. Several strongly continuous relations were broken e.g. the negative relation between VLDL and LDL.

9 The concentrations of VLDL and LDL as well as the composition of these LP were used in order to discuss possible pathogenetic mechanisms and metabolic blocks in the 5 different types of HLP Utilizing present metabolic concepts concerning LP metabolism it was possible to arrive at different lesions in the metabolism of LP Type II A II B III and V HLP could thus be explained

by single metabolic abnormalities while type IV HLP by virtue of its great heterogeneity from the LP compositional point of view could be divided into several subgroups with different pathogenesis.

10 Clinical and laboratory abnormalities were most frequent in subjects with type IV HLP Subjects with this type of HLP had higher body weights than controls. Type IV subjects had higher uric acid and S-GPT levels.

11 Multiple tendon xanthomata were exclusively seen in type II A palmar xanthomata in the presence of β-VLDL and eruptive xanthomata in one case with type V HLP Xanthelasmata and arcus corneae were found in several types of HLP and also in controls. However the presence of arcus was closely related to the concentration of LDL cholesterol.

12. In the resting ECG there were no differences between controls and HLP subjects with regard to Q-wave items and ST segment changes.

13 The frequency of ST segment depressions from Minnesota code 4 1 up to and including 4 4 during e ercise to near maximal heart rate was higher in all types of HLP than in controls. The frequency increased with age and was higher in females than in males. There was a tendency for the frequency to be higher in non-smokers than in smokers. There was no differences in frequency of ST depressions between subjects having low and high diastolic blood pressure and low and high glucose tolerance

14 The dependence of ST depression on LP concentrations and other factors was studied further by multiple regression analysis. The most signif cant determinant of ST depressions was age Of other to age singly added factors the following LPs were significant determinants VLDL cholesterol ($p<0$ 05 females) VLDL TG ($p<0.05$ males $p<0$ 02 females) LDL cholesterol ($p<0.001$ $p<$ 0 05) LDL TG ($p<0$ 01 $p<0$ 02) HDL did not contribute significantly Neither smoking, diastolic blood pressure or iv glucose tolerance made any significant contribution to the probability of ST depressions. Upon age the only non-lipid "risk factor" significantly increasing the probability of ST depressions was ESR in females ($p<0$ 05)

15 When different LPs were studied in the same equation in addition to age and LDL cholesterol only VLDL TG in men significantly increased the risk for ST depressions. Therefore tables were constructed giving the probability of having ST depressions during exercise based on age LDL cholesterol and VLDL TG.

Multiple logistic regression analysis gave similar results and did not improve the fitting of the equations

16 The physical working capacity was lower in most types of HLP This could be explained by a lower degree of physical training, genetic factors or a less ffective myocardial function in HLP

17 Signs of arterial disease of the lower limbs as estimated by the inclination time at digital pulse plethysmography were found in about 10 per cent of the HLP subjects. This was seen most often in type II B and III least often in type II A. Elevation of LP TG either in VLDL or LDL, was the most constant LP abnormality in connection with prolonged inclination time I addition almost all of these subjects were smokers and above 50 year of age.

18 ESR was higher in all types of HLP than in controls. This was interpreted as an expression of silent vascular disease caused by HLP

GENERAL CONCLUSION

Asymptomatic subjects who at a health control were distinguished only by hyperlipoproteinaemia showed a higher frequency of ST segment depressions during exercise suggesting a higher prevalence of subclinical coronary artery disease than subjects with normal serum lipid levels. Multiple regression analysis showed that particularly the concentration of LDL cholesterol and also that of VLDL TG were "independent predictors" of exercise ST segment depressions. This suggests that single or combined elevation of these lipoproteins hasten the development of coronary artery disease

Subclinical signs of peripheral artery disease were most frequent in connection with moderate elevations of TG either in VLDL or LDL, but were *not seen in subjects with extreme elevation of LDL cholesterol.*

These findings might suggest that LDL predominantly exerts its atherogenic potency in the coronary arteries while VLDL seems to be atherogenic to both coronary and peripheral arteries.

Quantitative lipoprotein analysis with scrutinization of lipoprotein concentrations compositions and interrelations showed that the currently used typing system for hyperlipoproteinaemia – although still useful – seems arbitrary and artificial Analysis of the lipoprotein characteristics of the different types revealed a pattern suggesting specific metabolic blocks or changes in turnover rates as mechanisms underlying the different types of hyperlipoproteinaemia.

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Incidence of Chronic Renal Insufficiency

A study of the incidence and pattern of renal insufficiency in adults during 1966–1971 in Gothenburg

By Jarl Ahlmén

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Acta Medica Scandinavica

originally published as *Nordiskt Medicinskt Arkiv* was founded in 1869 by Professor Axel Key MD. In 1901 (from volume 34) this journal was divided into a medical and a surgical section. Since 1919 (from volume 52) the medical section has been published under the name of *Acta Medica Scandinavica*

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CONTENTS

INTRODUCTION

The first estimates of the number of deaths from uremia appeared a few years after the effectiveness of the methods of treatment of the uremic state became evident. The first successful clinical application of hemodialysis took place in 1943 (46) but it was not until 1960 (70) that it became possible to maintain long-term hemodialysis of patients. Human kidney transplantation was first successfully performed between genetically identical persons in 1954 (61). Transplantations between non-identical persons could not be performed (35 58) until effective immunosuppressants to postpone graft rejection were in clinical use (74 18, 81). The improved results of both dialysis and transplantation increased the need for facilities for the care of uremic patients. A steadily increasing number of patients was treated (30 86). Increasing experience on the limitations of both methods led to a more intimate relationship between maintenance hemodialysis and kidney transplantation compared to the initial rivalry (71 75) in the care of patients with chronic uremia.

Above all, any calculations of the need for treatment facilities have to be based on a knowledge of the number of patients who develop terminal renal insufficiency each year (25 26 17). The awareness of a discrepancy between the number of patients referred to active treatment of chronic uremia and the number of patients succumbing from uremia (1 76 66) made planning of facilities for treatment very difficult. Any calculations have to rely on the results attained with active treatment, which in turn are dependent on the selection of patients. Improved results of treatment will reduce the mortality (28 47 85) and also broaden the indications for treating uremic patients with dialysis and kidney transplantation. As both dialysis and transplantation are expensive methods of treatment the economic impact on the welfare programmes of the community will be increasingly heavy. The problems for both the medical and public health profession will remain great (67) until preventive measures influencing the various causes of the renal diseases leading to progessive renal failure decrease the number of patients in need of active treatment.

The first investigations on mortality from uremia concentrated on calculation of the need for chronic dialysis. Death certificates were the source of estimates of the number of deaths in terminal renal insufficiency (88, 84 42, 22). As death certificates might conceal a number of patients with chronic renal disease some investigations have included a retrospective follow-up study of a sample of all deaths in a specific area concerning especially deaths from uremia. This was done by visiting and questioning relatives of the deceased and by scrutinizing hospital records to find indications or contraindications for active treatment of uremia (50). Other investigators used hospital records as the only source for calculations of the frequency of deaths from uremia (2 32). Estimates have also been made by comparing death certificates with hospital records (92). A comparison of the results obtained from death certificates with morbidity studies revealed that for the estimate of chronic maintenance dialysis the death certificates would reveal about 90 per cent of suitable candidates for active treatment (59). Merely recording the number of patients with renal failure admitted to a medical clinic and comparing them to other patients admitted during a certain follow-up period (77) does not however reflect the true situation concerning chronic uremia. There have been other methods using questionnaires sent to all physicians in a specified area (60, 78) but their reliability depends on the number of physicians replying. More systematic prospective

investigations including analysis of biochemical data at a hospital laboratory hospital records and questionnaires to practitioners in a defined area will decrease such methodological errors (13 56).

The only way to obtain an accurate impression of the progressive course of a chronic disease is by comprehensive epidemiological population studies with frequent follow-up periods (53). Population studies are however time-consuming, expensive laborious and too slow when a new effective method of treatment is developing. In order to obtain a complete understanding of the course of chronic renal failure to uremia the number of persons in such an investigation of renal diseases must be rather large as the prevalence of uremia is relatively low Studies of mortality in uremia combined with morbidity studies of renal diseases might be a fairly rapid method of discerning constant trends of the incidence of chronic renal failure

PURPOSE

The aim of the present investigation was to study chronic renal diseases in the city of Gothenburg during a six-year period. Special attention was paid to determine the incidence of azotemia, the rate of progress of renal disease and the incidence of uremia.

The present study did not aim to cover the ages 0–15 years as very few patients in this age-group suffer from renal diseases leading to uremia, nor was the aim to cover ages above 75 years as very few patients in these ages with renal diseases are suitable for treatment with dialysis and transplantation. Therefore the study was restricted to persons in the age group 16–75 years.

DEFINITIONS

Azotemia

All patients with decreased renal function leading to an increase of creatinine and urea in the blood are azotemic. In this study the term azotemia was restricted to define a state with a serum creatinine of 5 mg/100 ml or above.

Transient azotemia implies a period of increased serum creatinine above 5 mg/100 ml without any further period of increase above this value during the following year.

Pre-uremic state was used for patients with severe azotemia but without toxic symptoms of uremia.

Terminal uremia

Terminal uremia was defined as the state of uremia when toxic symptoms appeared or the patient died from uremia.

Rate of progress of renal disease

The rate of progress of renal disease was determined from the estimated date when a serum creatinine of 5 mg/100 ml was noted until the time of death from uremia or the time for institution of dialysis or transplantation because of uremia.

Hypertension

Hypertension was defined as a diastolic blood pressure of ≥ 105 mm Hg on at least two occasions. Malignant hypertension was defined as a diastolic blood pressure of at least 130 mm Hg in combination with retinopathy with papilloedema.

METHODS

Method of investigation

All diagnoses of diseases that might affect the renal function and lead to chronic azotemia were listed according to the "International Classification of Diseases" accepted by WHO in 1966. Moreover some additional diagnoses of diseases were listed that might influence the renal function for shorter periods of time. This was done to exclude the possibility that a chronic renal disease might be concealed under a misleading heading. The nomenclature was changed during the investigated years 1966–1971. The investigated diagnoses and the changes of the nomenclature for 1966 and 1971 are demonstrated in Table I. In Sweden an additional sub-classification, marked with two decimals, is used. The classification was also changed during the period and Table I illustrates some examples of these changes.

The computerized register of diagnoses of patients treated in hospital in Gothenburg was used to identify patients with diseases that might lead to chronic azotemia. The university hospital (Sahlgren's) is the major hospital where the majority of the patients were treated. The archives of case records in this hospital comprise most patients treated in hospital in Gothenburg. The archives of two minor hospitals (Ekman's and Carlander's Hospital) as well as the archives of two hospitals for chronic diseases (Hogsbo and Vasa Hospital) were included in the search for the case records of azotemic patients.

Diagnosis of the renal disease was determined from the case history, laboratory findings and histopathological examination.

The serum creatinine level of 5 mg/100 ml was chosen to reduce the number of periods and thus the number of patients with transient azotemia because of postrenal hindrance, dehydration, etc as far as possible.

The progress of renal insufficiency varies between different renal diseases. In order to investigate the rate of progress to uremia the date for serum creatinine 5 mg/100 ml had to be estimated. Four determinations of serum creatinine, 2 determinations within one year before and 2 determinations within one year after the level of 5 mg/100 ml, were required to delineate a curve for determination of the date for the value of 5 mg/100 ml. At least two determinations had to be made within a six month period including the estimated date of the serum creatinine of 5 mg/100 ml.

Hypertension, phenacetin abuse and urinary tract infection were especially looked for as these factors might be important as regards influencing the progress of renal disease and consequently the incidence of renal failure.

Determination of creatinine

Blood sampling for determination of creatinine in serum was performed in the morning with the patient fasting. The laboratory determination of serum creatinine was performed at the chemical laboratory on a Technicon Auto-Analyser (83). This method was not changed during the period of the investigation.

Statistical methods

Statistical comparisons were made using Fisher's permutation test (65) and sign tests (21).

Table 1 *List of diagnoses used for the search for azotemic and uremic patients.*

1966		1971	
016	Tuberculosis uro-genitalis	016	Tuberculosis uro-genitalis
		189	Neoplasma malignum organorum urinariorum aliorum et NUD
180	Neoplasma renis	223	Neoplasma benignum renis et organorum urinariorum aliorum
		237	Neoplasma non definitum uro-genitalium aliorum
181.01	Neoplasma vesicae urinariae malign.	188.99	Neoplasma malignum vesicae urinariae
260	Diabetes mellitus	250	Diabetes mellitus
288	Diathesis urica	274	Diathesis urica
289 10	Amyloidosis universalis	276.99	Amyloidosis universalis
?96 14	Purpura allergica Henoch-Schonlein	287 00	Purpura allergica Henoch-Schönlein
441.99	Morbus cordis hypertonicus essentialis malignus	400	Hypertonia maligna
445.99	Hypertonia essentialis maligna (morbo cordis sive nephrosclerosi exceptis)		
		403.99	Hypertonia essentialis cum morbo renis
446.99	Hypertonia cum nephrosclerosi arteriosclerotica	404.99	Hypertonia essentialis cum morbo cordis et renis
		440.10	Arteriosclerosis renis
		440.38	Ateriosclerosis alia
		444.30	Embolia et thrombosis arteriarum renis
456.20	Lupus erythematodes disseminatus	734 10	Lupus erythematodes disseminatus
590 10	Nephritis acuta	580.99	Nephritis acuta
591 99	Nephrosis	581.99	Nephrosis
59_07	Uraemia c nephritis chronica	582.00	Nephritis chronica cum uraemia
59 .99	Nephritus chronica UNS	582.09	Nephritis chronica NUD
593 99	Nephritis, casu acuto vel chronico non indicato	583.99	Nephritis NUD
594 98	Nephrosclerosis alia		
600	Morbi infectiosi renum	590	Infectio renis
60	Calculus renis et ureteris	592	Calculus renis et ureteris
603	Alii morbi renis et ureteris	593	Morbi renis et ureteris alii
		595	Cystitis
757	Maleformationes congenitae organorum urogenitalium	753	Maleformationes congenitae organorum urinariorum
786.50	Oliguria	786.50	Oliguria
786.51	Anuria	786.51	Anuria
79_99	Uraemia	792.99	Uraemia

MATERIAL

A total number of 8 271 patients were registered under the diagnoses listed in Table I. A screening was performed of 8 174 case records. The remaining 97 case records (1.2 per cent) could not be found in the case record archives of the various hospitals. Out of 8 174 case records of patients with the investigated diagnoses, 7 668 case records did not fulfil the criteria for inclusion in the investigation. The remaining 506 case records fulfilled these criteria and were used for analysis.

Information on the population of Gothenburg was collected from the Statistical Year Book for Gothenburg. The variations in the population during the period investigated may be seen from Table II. The age and sex distribution in 1966 and 1971 is given in Fig. 1.

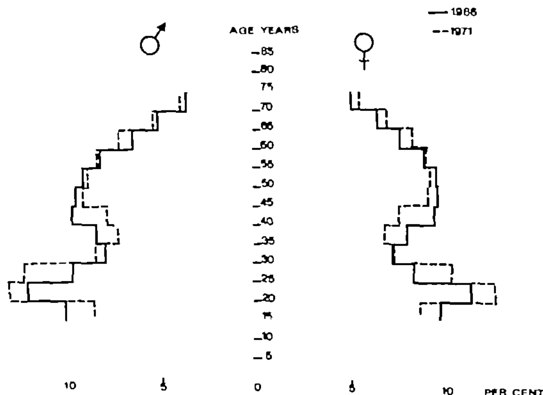


Fig. 1 Age and sex distribution in per cent of the population between 16 and 75 years of age in 1966 and in 1971 in the city of Gothenburg.

Table II. *Total population in the age groups 16–65 and 16–75 years in the city of Gothenburg during the period of investigation according to the Statistical Year Book for Gothenburg.*

		Number of inhabitants		
		Age	Age	
Year	Date	16–65	16–75	All ages
1966	1/11	289 654	322 094	425 108
1967	1/11	301 623	335 320	444 202
1968	31/12	300 274	334 885	443 678
1969	31/12	302 179	337 443	446 071
1970	31/12	305 801	341 841	450 860
1971	31/12	303 957	340 671	448 792

The investigation was restricted to adults between 16 and 75 years of age. An upper age limit of 65 years was also used in the calculations as this age limit, with a few exceptions, includes most patients with renal failure suitable for active treatment with dialysis or kidney transplantation at the present time.

RESULTS

A total number of 506 case records of patients with creatinine values of ≥ 5 mg/100 ml serum were found, 281 men and 225 women. Included were all persons between 16 and 75 years of age known to have had azotemia with a serum creatinine of ≥ 5 mg/100 ml, all deaths within these ages from renal failure as well as all patients actively treated for terminal renal failure within the city of Gothenburg included. The azotemia was due to extrarenal causes in 79 cases. Renal disease was the cause of azotemia in 427 cases (Table III). In 58 of these 427 patients the azotemia was developing before 1966. For the remaining 369 patients the azotemia developed during the years investigated.

Table III *Total number of case records showing a serum creatinine level of ≥ 5 mg/100 ml during 1966–1971*

Azotemia due to extrarenal causes		79
Azotemia due to renal disease		427
Azotemia developing before 1966	58	
Azotemia developing 1966 1971	369	
Total number of azotemic patients		506

Incidence of azotemia

AZOTEMIA DUE TO EXTRARENAL CAUSES

In 79 patients, 54 men och 25 women the azotemia was due to extrarenal causes. As shown in Table IV 52 patients had acute renal insufficiency due to prerenal factors as the cause of azotemia and 27 patients suffered from acute post renal obstruction. Distribution of the patients per year was 9 13 15 9 18 and 15 patients respectively from 1966 to 1971 As these patients did not contribute to chronic renal failure they are not included in the following calculations.

AZOTEMIA DEVELOPING BEFORE 1966

Fifty-eight patients reached their first increase of serum creatinine above 5 mg/100 ml before 1966 and 22 of them before 1965 These 58 cases were not included when determining the incidence of azotemia of renal disease during the period investigated but 51 of them were included in the figures for terminal uremia for the period. Five patients with chronic pyelonephritis died from causes other than uremia – 1 from myocardial infarction (72 years old) 1 from intoxication (38 years old), 1 (72 years old) from circulatory insufficiency 1 (60 years old) from cerebral hemorrhage and 1 patients (75 years old) died after 1971 Two patients are still alive (December 1974). They had a transient azotemia because of intermittent obstruction from necrotic material of renal papillae in the course of chronic pyelonephritis with papillary necrosis.

Table IV *Patients with acute azotemia due to extrarenal causes*

	No of patients	No of patients surviving
Intoxication	13	11
Postoperative	8	3
(Res. ventriculi, abdom. tbc ileus, aortic aneurysm (?) thrombendarterectomy V.O.C aortae neurosurgical op)		
Unknown causes	6	5
Hepatorenal syndrome	5	2
Dehydratio	4	2
Pancreatitis	3	2
Sepsis	2	2
Cerebral haemorrhage	2	
Neoplasma malign. mammae	2	
Rupture of aortic aneurysm	2	
Cholecystitis	1	1
Multiple trauma	1	1
Myocardial infarction	1	
Pulmonary embolism	1	
abscess	1	
Malignant disease		
Neoplasma vesicum	8	4
prostatae	5	1
caeci	1	
uteri	1	
ovarii	1	
Lymphosarcoma	1	
Lymphoma	1	
Non malignant	9	7
Total no	79	41

AZOTEMIA DEVELOPING DURING 1966–1971

Azotemia appeared in 369 patients aged 16–75 years during 1966–1971. The age and sex distributions among these 369 patients are shown in Fig [illegible]. The number of patients and ages during each year of the period are shown in Tabel V. The number of [illegible] appearing [illegible] 1966 was 67. A maximum number [illegible] 0 was found in 1968 and after this a decreasing trend appeared with a minimum number of [illegible] patients [illegible]. The changes during the years [illegible] the [illegible] diseases may be followed in Table VI. The changes were relatively small for all diseases except for pyelonephritis. The age group 16–45 comprises 18 per cent of the total number of patients. The age group 46–65 years included 51 per cent of the patients. Thirty-one per cent of the 369 patients were aged above 65 years.

The most common renal diseases in the patients included in the incidence figures for azotemia were pyelonephritis, glomerulonephritis, diabetic nephropathy, nephrosclerosis and polycystic kid-

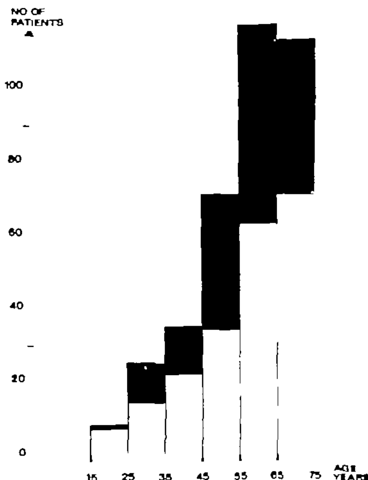


Fig. 2. Age and sex distribution of 369 patients with azotemia. The unfilled areas represent men and the shaded areas women.

ney disease (Table VI). These five diseases comprised 315 of the total number of 369 patients i.e. 85 per cent of the incidence of azotemia.

The age and sex distributions for these five diseases are shown in Fig. 3. A male preponderance was found in the glomerulonephritic group (61/16 male/female) and a female preponderance in the pyelonephritic group (54/90 = male/female). A male preponderance was also seen among patients with nephrosclerosis (20/5 = male/female). The group of patients with nephrosclerosis showed an age maximum between 66 and 75

Table V *Number of patients with azotemia according to age during the period investigated.*

Age	16–25	26–35	36–45	46–55	56–65	66–75	Total
1966	3	8	7	11	21	17	67
1967	1	5	8	13	21	19	67
1968	2	4	5	13	23	23	70
1969	0	1	6	11	22	22	62
1970	1	5	5	16	12	13	52
1971	1	2	4	7	18	19	51
Total	8	25	35	71	117	113	369

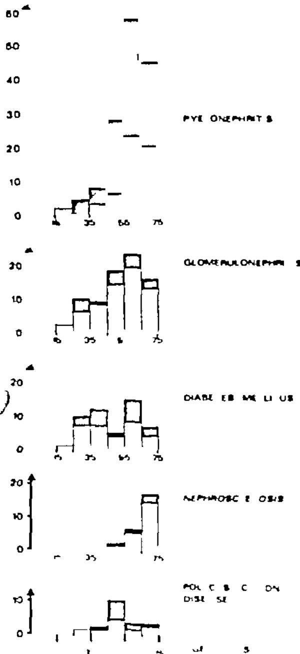


Fig 3 Distribution of ge and sex according t renal disease among 315 p tients w th py lonephritis, glomerulonephritis, diabetes, ephrosclerosis nd p kidney disease The unfa d area refer shaded areas refer t women

years. Glomerulonephritis patients and especially patients with pyelonephritis were most often aged between 56 and 65 years. The age distribution among patients with diabetic nephropathy showed two maxima, one between 36 and 45 years and the other between 56 and 65 years. The patients with polycystic kidney disease were most often azotemic when aged between 46 and 55 years.

The number of patients with transient azotemia included in the incidence figures was 32 Distribution over the years showed (Table VII) that these patients especially influenced the incidence figures for 1967 1968 and 1969 while the figures for 1971 were relatively unaffected. Pyelonephritis was the most common disease among patients with transient azotemia.

The total incidence of azotemia each year depends on the annual variations of the renal diseases. In order to reduce the influence of these annual variations comparison between two three -year periods was chosen for the calculations of incidence The number of azotemic patients in 1966 1967 and 1968 decreased significantly from 204 to 165 azotemic patients in 1969 1970 and 1971 ($p < 0.05$).

The mean incidence for the period was 183 azotemic patients per year and million inhabitants. An incidence of 208 200, 209 184 15? and 150 azotemic patients per million inhabitants was found for each of the years 1966 to 1971 respectively

When the upper age limit was reduced to 65 years the total number of azotemic patients was 258 The number of azotemic patients was 50 in 1966 decreasing to 33 in 1971 The incidence of azotemia showed a decreasing tendency as shown in Fig 4

The cause of azotemia at ages 16–65 years was most often pyelonephritis, glomerulonephritis and diabetes mellitus (Table VIII) When the first three years of the period were compared to the last three years the number of azotemic patients decreased significantly from 146 patients to 112.

The mean incidence for the six-year period was 143 azotemic patients per year and million inhabitants In 1966 the incidence of azotemia at ages 16–65 years was 173 patients per million inhabitants decreasing to 109 patients per million inhabitants in 1971 (Fig 5)

Table VI. *Distribution of renal diseases among the 369 azotemic patients aged 16–75 years during the years 1966–1971*

	1966	1967	1968	1969	1970	1971	Total
Pyelonephritis	31	30	29	18	17	19	144
Glomerulonephritis	10	13	14	12	15	13	77
Diabetes mellitus	12	10	6	9	6	7	50
Nephrosclerosis	4	5	4	8	3	1	25
Polycystic kidney disease	3	2	6	4	2	2	19
Amyloidosis	1	2	4	3	4	3	17
Systemic lupus erythematosus	1	1	1	0	1	2	6
Other diseases	5	4	6	8	4	4	31
Total	67	67	70	62	52	51	369

Table VII. *Number of patients aged 16–75 years with transient azotemia during the years investigated*

	1966	1967	1968	1969	1970	1971	Total
Pyelonephritis	2	1	4	5	1		13
Glomerulonephritis		3	1	1			5
Diabetes mellitus	3		1		1	1	6
Nephrosclerosis					1		1
Polycystic kidney disease					1		1
Other diseases		3	1	1	1		6
Total	5	7	7	7	5	1	32



Fig. 4 Number of azotemic patients aged 16–65 years during the period 1966–1971

Table VIII *Distribution of renal diseases among the 258 azotemic patients aged 16–65 years during the years 1966–1971*

	1966	1967	1968	1969	1970	1971	Total
Pyelonephritis	22	18	18	16	11	14	99
Glomerulonephritis	9	[illegible]	13	7	12	8	61
Diabetes mellitus	10	8	6	8	6	5	43
Polycystic kidney disease	2	[illegible]	4	4	2	2	16
Amyloidosis	1	2	3	2	4	1	13
Nephrosclerosis	2	2	1	1	[illegible]	0	8
Systemic lupus erythematosus	1	1	1	0	1	[illegible]	6
Other diseases	3	3	2	2	1	1	1
Total	50	48	48	40	39	33	258

Pyelonephritis

One hundred and forty four patients aged 16–75 years had pyelonephritis as the cause of azotemia. In 2 patients no histopathological confirmation of the diagnosis was available. The pyelonephritic group of patients was divided into three sub-groups: one sub-group (I) of 7 patients with obstructive pyelonephritis, one sub-group (II) of 71 patients with non-obstructive pyelonephritis and one sub-group (III) of 66 patients with phenacetin-induced nephropathy (Table IX). Pyelonephritis was used as a common term for all three sub-groups.

Thirteen patients had periods of transient azotemia (Table VII). The cause of the transient azotemia was in 8 patients obstructive necrotic papillae, in 2 patients it was probably caused by postoperative tubular necrosis, in 1 patient by urinary retention and in 2 patients it was probably due to dehydration.

In the non-obstructive sub-group of 71 patients papillary necrosis was proven in 11 patients but only 2 of them admitted irregular phenacetin abuse. The case records of 5 patients showed no record of phenacetin abuse and the remaining 4 patients denied phenacetin abuse. Clinical suspicion of phenacetin abuse existed in some cases but was not proven. There was a female preponderance ([illegible]9/42 = male/female). On comparing the first three years (1966, 1967 and 1968) with the later three years (1969, 1970 and 1971) some differences were revealed. Periods of transient azotemia were not included in any of the following calcula-

Fig. 5. Incidence of azotemia of ages 16–65 years in the city of Gothenburg during each year

Table IX. *Distribution of patients aged 16–75 years with pyelonephritis according to sub-groups (see text)*

	1966	1967	1968	1969	1970	1971	Total
Pyelonephritis							
Sub-group I	2		4	1			7
II	19	16	14	4	7	11	71
III	10	14	11	13	10	8	66
Total	31	30	29	18	17	19	144

tions. There was a decrease in sub-group II from 47 to 24 patients in the age group 16–75 years. Women decreased significantly in numbers from 29 to 13 i.e. 55 per cent while the decrease in men was 39 per cent. If the 11 patients with proven papillary necrosis are accepted as probable phenacetin abusers and included in sub-group III there was a total decrease in number from 39 to 21 patients from the first three years compared to the last three years in sub-group II. Women decreased significantly from 24 to 11 i.e. a decrease of 54 per cent while corresponding decrease for men was 33 per cent.

Patients referred to the sub-group with phenacetin nephropathy had a history of consumption of three or more doses of phenacetin-containing drugs a day during five years. Papillary necrosis was seen in all but 5 of 66 patients with phenacetin nephropathy either upon histopathological investigation or in the form of typical changes on radiological investigation (49). There was a female preponderance (20/46 = male/female). When comparing the number of patients from the first three years with the patients of the last three years of the period a total decrease from 39 patients to 27 was seen. A significant decrease from 31 to 15 women was found while there was a change from 8 to 12 men between the two periods. When the 11 patients with papillary necrosis transferred from sub-group II were taken into account the female decrease in sub-group III was still significant, from 36 to 17 patients while for the male patients a change from 11 to 13 was found.

Analysis of the incidence of pyelonephritis in younger and elderly patients was of special interest. When comparing the age groups 16–55 and 56–75 among the patients in the non-obstructive sub-group it was found that 20 per cent of the patients were included in the former and 80 per cent in the latter age groups. Four men out of 29 and 10 women out of 42 were younger than 56 years. Using the same age grouping among the patients in the sub-group with phenacetin nephropathy resulted in 41 per cent of the patients being younger than 56 years. Seven out of 20 men and 20 out of 46 women were younger than 56 years. If the 11 probable phenacetin abusers with proven papillary necrosis were transferred from sub-group II to

Table X. *Distribution according to age groups 16–55 and 56–75 of azotemic patients with pyelonephritis in sub-group II and sub-group III (see text).*

	Age Years	1966	1967	1968	1969	1970	1971	Total no.
II	16–55	4		1		1	3	9
	56–75	13	12	9	6	3	8	51
III	16–55	7	6	6	3	7	3	32
	56–75	5	14	9	6	6	5	45

sub-group III an even more marked age difference between the two sub-groups was found (Table X). This meant that the sub-group with phenacetin nephropathy became greater (77 patients) than the sub-group with non-obstructive pyelonephritis (60 patients). The patients with mainly phenacetin nephropathy reached a serum creatinine value of 5 mg/100 ml at an earlier age than patients in the non-obstructive sub-group with pyelonephritis.

The significant decrease among women at ages 16–75 in the sub-group with non-obstructive pyelonephritis and in the sub-group with phenacetin nephropathy was of the same magnitude. In both sub-groups the decrease in the number of women was most apparent in the age group 56–75 years. A maximum incidence of 31 pyelonephritic patients with azotemia aged 16–75 years was found in 1966 and a minimum of 17 in 1970. There was a significant decrease ($p < 0.05$) of azotemic patients with pyelonephritis when the number of patients in the first years were compared to the number of patients in the last three years. The mean incidence of pyelonephritic patients aged 16–75 years with azotemia was 7? cases per year and million inhabitants during the period 1966–1971.

In the age group 16–65 years the incidence of azotemia because of pyelonephritis showed a decreasing tendency as shown in Fig. 6. Exclusion of patients with transient azotemia reduced the number of patients with cronic azotemia developing during 1966–1971 to 91. However 5 patients with transient azotemia before 1966 developed chronic azotemia during the six year period of

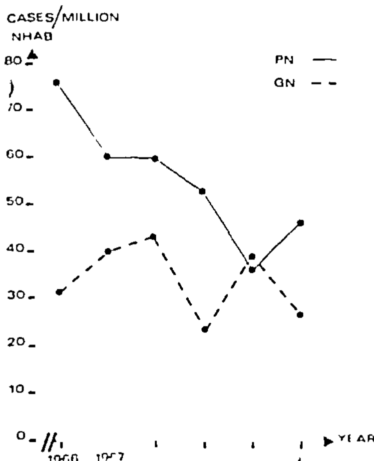


Fig. 6. Incidence per yea of azotemia in patient aged 16–65 year with pyelonephritis (PN) and glomerulonephritis (GN) per mill. inhabit.

investigation. The total number of patients with chronic azotemia due to pyelonephritis from 1966 to 1971 was 96 (Table XI). The incidence of chronic azotemia per year and million inhabitants is shown in Fig. 7. The mean incidence of chronic azotemia for the period was 53 cases per year and million inhabitants. A decreasing tendency was found, from 73 cases per million inhabitants in 1966 to 46 cases per million inhabitants in 1971.

Hypertension. Twenty-five patients (17 per cent) showed a creatinine value of more than 5 mg/100 ml when first admitted to hospital and their previous history of hypertension at a creatinine level of 5 mg/100 ml serum could not be evaluated. Among the remaining 119 patients of the 144 with pyelonephritis, 24 men and 40 women had hypertension. Twenty men and 35 women did not have hypertension.

Table XI. Distribution of patients aged 16–65 years with chronic azotemia due to pyelonephritis according to sub-groups (see text).

	1966	1967	1968	1969	1970	1971	Total
Pyelonephritis							
Sub-group							
I	1		2	1			4
II	11	6	8	4	5	10	44
III	9	11	9	8	7	4	48
Total	21	17	19	13	12	14	96

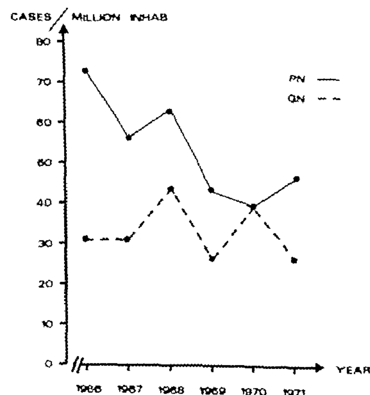


Fig. 7. Total number of cases of chronic azotemia with pyelonephritis (PN) or glomerulonephritis (GN) per million inhabitants aged 16–65 years.

Urinary tract infection. Complicating factors made the history of urinary tract infection less valuable in 8 patients. In another 22 patients the history was incomplete. A total of 114 patients remained for evaluation. No history of urinary tract infection and no bacterial growth with more than 100 000 bacteria/ml on urinary cultures was found in 3 patients out of 51 patients in the sub-group with non-obstructive pyelonephritis. One of these 3 patients showed papillary necrosis at necropsy. Recurrent urinary tract infections were present in 32 of the remaining 47 patients.

No urinary tract infection was found among 11 out of 57 patients included in the sub-group with phenacetin nephropathy. Recurrent urinary tract infections were found among 22 of the remaining 46 patients in this sub-group.

Glomerulonephritis

Glomerulonephritis was the cause of azotemia in 77 patients in the age group 16–75 years. No histopathological confirmation of the clinical diagnosis was available in 0 patients. Twenty-nine out of 61 [illegible] and 9 out of 16 women were younger than 56 years. The number of patients changed irregularly from year to year (Table VI). Five patients with transient azotemia were included (Table VII). Two of these patients had a transient azotemia due to acute glomerulonephritis. Both (35 and 19 years old) lived four years later with a normal serum creatinine. Two of the remaining patients with transient azotemia appeared later during the period with chronic azotemia. Another patient (49 years old) with transient azotemia which developed before 1966 had chronic azotemia in 1969. The annual change of the incidence of chronic azotemia because of glomerulonephritis was 10 10 14 13 15 and 1[illegible] patients. This meant a total mean value of 37 cases per year and million inhabitants of chronic azotemia due to glomerulonephritis at ages 16–75 years.

The number of patients aged 16–65 years varied irregularly from year to year as shown in Table VIII. Th[illegible] [illegible] during the years of the period per million inhabitants showed no decreasing or increasing [illegible] (Fig 6). All 5 patients with transient azotemia were younger than 66 years and when they were taken [illegible] some small changes in the incidence accurred (Fig 7) but did not change the impression of a constant incidence of glomerulonephritis during the years. A mean incidence of 33 cases per year and million inhabitants at ages 16–65 years was found.

Hypertension. Nineteen (25 per cent) of the 77 patients with glomerulonephritis had a serum creatinine above 5 mg/100 ml when first admitted to hospital. A previous history of hypertension in these 19 patients at a creatinine level of 5 mg/100 ml could not be evaluated. Hypertension was registered in 31 men and 8 women. One male patient (67 years old) showed for some time hypertension that could be clearly related to immunosuppressive treatment. His blood pressure returned to normal values when immunosuppressive treatment was interrupted and he was included in the group of non-hypertensive patients consisting of 14 men and 5 women.

Diabetes

Diabetic nephropathy was the cause of azotemia in 50 patients in the age group 16–75 years. Necropsy was not performed in 6 patients. Long-standing diabetes, diabetic retinopathy, heavy proteinuria and death from uremia characterized 5 of these patients. One patient (48 years old) had a history of diabetes for nine years, diabetic retinopathy for one year and heavy proteinuria indicating that the cause of uremia was a diabetic nephropathy. He also had a history of glomerulonephritis with edema and proteinuria about ten years before the onset of his diabetes. His ultimate diagnosis might have been a combination of glomerulonephritic and diabetic nephropathy.

The variation in numbers of azotemic patients because of diabetic nephropathy are shown in Table VI. A maximum number of 1[illegible] patients was registered in 1966. Six patients with diabetic nephropathy had a transient azotemia. One of these 6 patients later appeared with chronic azotemia. Three of them died from vascular causes during the period without any further increase of serum creatinine above 5 mg/100 ml and the remaining [illegible] patients had transient azotemia because of an acute pyelonephritis and a diabetic coma respectively. When these 6 patients were

accounted for the number of patients with chronic azotemia due to diabetic nephropathy was 9 10 6 9 5 and 6 patients for the years 1966 to 1971 respectively. This meant a mean incidence of chronic azotemia due to diabetic nephropathy of ..2 cases per year and million inhabitants at age 16–75 years.

Using an upper age limit of 65 years, the number of patients with azotemia due to diabetic nephropathy decreased from 50 to 43 (Table VIII). When patients with periods of transient azotemia were accounted for the number of patients with chronic azotemia was 8, 8 6 8 5 and 5 during each of the six years respectively. Diabetic nephropathy was the cause of chronic azotemia at ages 16–65 years in on average 22 cases per year and million inhabitants during the period 1966 to 1971.

Hypertension was registered in 16 men and 14 women at a serum creatinine level of 5 mg/100 ml. No hypertension was found in 15 men and 5 women.

Nephrosclerosis

Nephrosclerosis was the cause of azotemia in 25 patients in the age group 16–75 years. Histopathological diagnosis supported the clinical diagnosis in 2.. patients. One patient (60 years old) had transient azotemia postoperatively. He was alive four years later with an almost normal serum creatinine. The remaining .. patients (70 years and 67 years old) had received medical treatment for hypertension for 20 and 15 years respectively. The most probable diagnosis was nephrosclerosis. Only 5 patients out of 25 were women. Patients aged above 65 years represented 68 per cent of the total 25 patients. Only 1 patient was below 55 years of age. The exception was a 48-year-old woman with malignant nephrosclerosis. The azotemic patients were unevenly distributed over the different years investigated (Table VI) but the material was too small to be conclusive.

Nephrosclerosis was found to be the cause of azotemia at ages 16–75 years in on average 12 cases per year and million inhabitants. Only 1 patient showed a transient azotemia after an operation. The age group 16–65 years comprised only 8 azotemic patients (Table VIII).

Hypertension. Four patients (16 per cent) were not known at hospital before being admitted for the first time and exhibited serum creatinine levels above 5 mg/100 ml. All 4 were dead within eleven days and it was not possible to get a reliable previous history concerning hypertension. Twenty of the other 21 patients had hypertension. The exception was a man (68 years old) with cardioarteriosclerosis and nephrosclerosis verified at necropsy. Eyeground investigations were not registered in the case records of 4 patients. One patient could not be examined ophthalmoscopically because of amaurosis and cataracts. Four patients had malignant hypertension. Another 5 patients exhibited exudate and haemorrhages at the ophthalmoscopic examination (Keith-Wagener-Barker III 38) but without papilledema.

Polycystic kidney disease

Polycystic kidney disease was the cause of azotemia in 19 patients in the age group 16–75 years. Only 1 patient had his renal disease diagnosed at a serum creatinine level above 5 mg/100 ml when first admitted to hospital. The diagnosis was in most cases known among the other 18 patients many years before the renal function had deteriorated to a serum creatinine level of 5 mg/100 ml. Six out of 9 men and 7 out of 10 women were younger than 56 years. The distribution during the years investigated showed a maximum number of 6 azotemic patients in 1968 (Table VI). One patient had a period of transient azotemia after an operation but did not develop chronic azotemia within the period of the investigation. Chronic azotemia due to polycystic kidney disease showed a mean incidence of 9 cases per year and million inhabitants at ages 16–75 years.

Hypertension. Five men and 6 women had hypertension out of the assessable 18 patients at serum creatinine 5 mg/100 ml. Three men and 4 women were normotensive.

Amyloidosis

In the age group 16–75 years amyloidosis was the cause of azotemia in 17 patients, 8 men and 9 women. A histopathological diagnosis was avail-

able in all cases. All patients but one had a chronic disease of extrarenal origin. Rheumatoid arthritis was present in 10 patients, ankylosing spondylitis in 2, tuberculosis in 3 – pulmonary in 2 patients and arthritic in one – and cronic respiratory disease in 1 patient. One patient (56 years old) had no previous history of chronic disease. His disease was suspected when he appeared with an epidermiolysis and was verified at necropsy ten months later when he died from circulatory insufficiency with a serum creatinine of 5.5 mg/100 ml. No family history was recorded and the family was not investigated further. Four patients were older than 65 years. No tendency of decreasing incidence was found during the years investigated (Table VI and VIII). Chronic azotemia due to amyloidosis showed a mean incidence of 8 cases per year and million inhabitants in the age group 16–75 years.

Systemic lupus erythematosus

All 6 patients with S.L.E. had their clinical diagnosis [illegible]enfied hist[illegible]pathologically. Four patients were women [illegible] 44, 48 and [illegible]0 years and [illegible] [illegible]n [illegible]1 and 45 years old.

Other diseases

The hete[illegible]enous group "other diseases" was the cause of az[illegible]temia in 31 patients in the age group 16–75 years. The various diagnoses are registered in Table XII.

Fi[illegible]e [illegible]f these pat[illegible]ents suffered from myelomatosis. One female p[illegible]tient (64 years old) was given the diagnosis [illegible] my[illegible]lomatosis on the basis of laboratory and [illegible]linical findings and was treated accordingly while the histopathological examination at nec[illegible]psy sh[illegible]wed [illegible] omplex picture with nephropathy in[illegible]luding thromb[illegible] arteritis and glomerular lesion suggestive [illegible]f periarteritis nodosa but no histol[illegible]gical e[illegible]idence of myeloma. Two patients with myelomatosis (63 and 71 years old) received peritoneal dialysis for one and one and a half months respe[illegible] [illegible] [illegible] [illegible]therapy. Both died at the end [illegible] the dial[illegible] [illegible]period. One patient (70 year[illegible] [illegible]ld) had been [illegible] a malignant tumou[illegible] of the urin[illegible] bl[illegible]dd[illegible] three years earlier whe[illegible] [illegible] also had [illegible] ally demonstrated pyel[illegible]ephrit[illegible] [illegible] had [illegible]neys. She did not a[illegible] any h[illegible] [illegible] abuse or urinary tract infections at that time. No histopathological diagnosis could be established.

Table XII. *Diagnoses of 31 azotemic patients with "other diseases."*

Malignant tumour	
renal	9
bladder	4
ureter	1
Myelomatosis	6
Leukemia myeloica	1
Wegener's granulomatosis	1
Periarteritis nodosa	2
Thrombosis art. ren. bilat.	1
Tuberculosis urogenitalis	1
Nephropathia	
Arthritis urica	1
Epidemica	1
NUD	3
Total no	31

when she died. One woman (72 years old) died ten days after admission from bilateral thrombosis of the renal arteries, the right artery probably having been occluded for a long time.

No necropsy was performed in 3 patients so that no definite pathological diagnosis of the renal disease was possible. They were classified as nephropathia NUD. One (60 years old) of these 3 patients had a ten-year history of hypertension and the other (74 years old) 14 years of known antihypertensive treatment. This male patient had a transient azotemia when he was operated on for hyperplasia of the prostata and his serum creatinine value returned to the preoperative value of 2.6 mg/100 ml. The third patient (55 years old) diagnosed as nephropathia NUD had intermittently received radiation therapy for Hodgkin's disease three to fi[illegible]e years before he died from uremia after a period of heavy nephrotic syndrome. Histologically it was not possible to differentiate between a membranous glomerulonephritis due to his original disease or a radiation nephritis. One patient (56 years old) suffered from a malignant tumour of the urinary bladder resulting in cystectomy and an ileal bladder substitute. After this operation he had recurrent urinary tra[illegible]t infections and repeated post renal obstructions of [illegible] ren[illegible] reuatus until he died from uremia three years

later. One patient (58 years old) was nephrectomized unilaterally because of nephrolithiasis 19 years before he developed a renal carcinoma in the remaining kidney. An unsuccessful extracorporal repair and autotransplantation of this kidney resulted ultimately in hemodialysis after removal of the transplant. The patient with Wegener's granulomatosis (55 years old) responded well to immunosuppressive treatment and his renal function improved to a serum creatinine level below 5 mg/100 ml. One male patient (36 years old) suffered from urogenital tuberculosis and he was treated actively with hemodialysis on a chronic basis. One patient with periarteritis nodosa (70 years old) died from uremia five days after he was admitted to hospital. Another patient (41 years old) with progression from a normal serum creatinine to uremia within one month received three peritoneal dialysis treatments before he died. Clinical and laboratory findings in favour of a generalized disease were verified pathologically with the diagnosis periarteritis nodosa.

Another 10 patients were dead within one year. Seven of these patients died from uremia, 1 from circulatory insufficiency, 1 from thrombosis of the mesenteric artery and 1 patient from cardiac arrythmia. The patient with nephropathia epidemica (37 years old) exhibited a transient azotemia with normalized serum creatinine within two weeks. Two patients (68 and 72 years old) had a transient azotemia after operation for hypernephromas. One patient (70 years old) with a for three years known hypernephroma with metastases had a transient azotemia during the course of myocardial infarction.

The number of patients included in the group "other diseases" seemed to be evenly distributed during the years investigated. When the 6 patients with transient azotemia were accounted for the incidence of chronic azotemia was 5, 1, 5, 7, 3 and 4 patients during the years 1966–1971 respectively at ages 16–75 years. If the upper age limit is reduced to 65 years there was a change from these numbers to 3, 0, 2, 2, 1 and 1 patient for the years 1966–1971 respectively.

Chronic azotemia and mortality

Of the patients with azotemia 300 patients in the age group 16–75 years included in the five largest diagnostic groups were followed up for 36 months (December 1974) in order to investigate differences in the causes of death between the groups. Pyelonehritis was the cause of azotemia in 134 patients, glomerulonephritis in 75 patients, diabetic nephropathy in 48 patients, nephrosclerosis in 25 patients and polycystic kidney disease in 18 patients. All patients with periods of transient azotemia were transferred to the year of chronic azotemia. If dialysis or transplantation was started during the follow-up period this patient was referred to the patients dying from uremia, i.e. he was included in the figures for the calculation of the mortality in uremia. A total of 308 patients were assessable with respect to the appearance of chronic azotemia but 8 of them were still alive at the end of the follow-up period (Table XIII). Seven out of these 8 patients suffered from pyelonephritis and in the remaining patient polycystic kidney disease was the cause of chronic azotemia.

Pyelonephritis. The main cause of death among the 134 azotemic patients with pyelonephritis was uremia, which was responsible for the death of 106 patients (79 per cent). Cardiovascular and cerebrovascular lesions were responsible for the death of 22 patients. Only 6 patients died from other causes. Two died from bronchopneumoniae, 1 from cancer coli with metastases, 1 from pancreatitis and cholecystitis, 1 from rupture of oesophageal varices and 1 patient from severe acidosis and circulatory insufficiency. Four of these 6 patients were older than 65 years. Totally 43 patients were older than 65 years. In the age group 16 to 65 years twelve per cent (11/91) of the patients died from cardiovascular or cerebrovascular causes while 26 per cent (11/43) of the patients died from these causes in the age group 66 to 75 years.

Glomerulonephritis. Sixty five patients (87 per cent) of the 75 patients with azotemia due to glomerulonephritis died from uremia. Cardiovascular and cerebrovascular lesions were responsible for the death of 7 patients, only 1 of whom was older than 65 years. Three patients died from pulmonary embolism, bronchial carcinoma and hepatic cirrhosis with acidosis respectively. Among the 75 patients 16 were older than 65 years. In the age group 16–65 years 10 per cent of the patients

Table XIII *Causes of death within the five largest groups of renal diseases followed up for 36 months. Distribution according to age groups 16–65 years and 66–75 years. "Uremia" signifies calculated mortality in uremia.*

	Age groups	Total no	Causes of death: Uremia	Cardio-vascular	Cerebro-vascular	Broncho-pneumonia	Other	Alive after 36 months observation time
Pyelonephritis	16–65	94	78	10	1	1	1	3
	66–75	47	28	8	3	1	3	4
	Total	141	106	18	4	2	4	7
Glomerulonephritis	16–65	59	50	4	2		3	
	66–75	16	15	1				
	Total	75	65	5	2		3	
Diabetes mellitus	16–65	42	35	3	2		2	
	66–75	6	1	3		1	1	
	Total	48	36	6	2	1	3	
Nephrosclerosis	16–65	8	5	1	1	1		
	66–75	17	10	3	1	2	1	
	Total	25	15	4	2	3	1	
Polycystic kidney disease	16–65	16	14				1	1
	66–75	3	3					
	Total	19	17				1	1

(6/59) died from cardiovascular and cerebrovascular causes

Diabetes. Thirty-six patients (75 per cent) of the 48 azotemic patients with diabetic nephropathy died from uremia. Cardiovascular and cerebrovascular lesions were the causes of death in 8 patients (17 per cent) One patient died from bronchopneumonia, 1 from perforation of the gall bladder with abscess development, 1 from multiple pulmonary embolism and 1 patient died in a metabolic acidosis of non-uremic origin Only 6 of the 48 azotemic patients with diabetic nephropathy were older than 65 years. In the age group 16–65 years 5 patients out of 42 died from cardiovascular and cerebrovascular causes

Nephrosclerosis. Fifteen (60 per cent) of the 25 patients with nephrosclerosis as the cause of chronic azotemia died from uremia. Four of the 5 patients with severe hypertension without papilledema at eyeground examination (Keith-Wagener-Barker III) died from uremia. The fifth patient died from myocardial infarction.

Four out of 5 patients with registered malignant hypertension during the course of the disease died from non-uremic causes. They died 6 months, 8 months, 19 months and 16 years after the onset of malignant hypertension. The fifth patient died in uremia 16 years after the diagnosis of malignant hypertension A total of 24 per cent of the 25 patients with nephrosclerosis as the cause of azotemia died from cardiovascular or cerebrovascular lesions. Three patients died from bronchopneumoniae and 1 patient from arterial embolism. Eight patients among the 25 patients with nephrosclerosis were younger than 66 years.

Polycystic kidney disease. All but 1 of the 18 azotemic patients with polycystic kidney disease succumbing during the three-year observation period died from uremia. Three patients were older than 65 years.

Rate of progress of renal disease

The rate of progress of chronic renal disease could be investigated in 143 patients. Pyelonephritis was the cause of chronic azotemia in 52 patients, glomerulonephritis in 40 patients, diabetic nephropathy in 24 patients, polycystic kidney disease in 11 patients, amyloidosis in 8 patients and nephrosclerosis in 8 patients (Tabel XIV).

The average rate of progress for all patients included was 10.8 months from the initial chronic azotemia to uremia.

Pyelonephritis

Fifty-two patients with non-obstructive pyelonephritis fulfilled the criteria for determination of the rate of progress until uremia. The mean duration from the initial chronic azotemia until terminal uremia for 52 patients with pyelonephritis was 13.6 months with a range of 0.7 to 52.4 months. Twenty-nine patients (56 per cent) reached terminal uremia within one year. A total of 19 patients (4 men and 15 women) were included in the non-obstructive sub-group of pyelonephritis (II) and 33 patients (11 men and 22 women) belonged to the sub-group with phenacetin nephropathy (III).

The rate of progress constituted for sub-group II a mean of 11.8 months and for sub-group III 14.7 months. There seemed to be a small difference between women in sub-group III with a mean time of 9.7 months and men with a mean time of 15.2 months. However transferring 5 probable phenacetin abusers with papillary necrosis from sub-group II to sub-group III reduced this difference in time from 5.5 months to 1.4 months.

Ten patients suffered a deterioration of renal function to terminal uremia within four months (Table XIV). One woman (64 years old) among these 10 patients progressed to terminal uremia in one month and a probable accelerating factor was a severe bronchial asthma with respiratory infection. Another woman (54 years old) with hypertension included in sub-group III had no special accelerating factor but her progress time was 0.7 months. Eight of these 10 patients were hypertensive but none had any signs of malignant hypertension during the course of the renal disease. Two *patients of the 31 with hypertension had malignant hypertension.*

Glomerulonephritis

The rate of progress to terminal renal insufficiency could be estimated in 40 patients with glomerulonephritis, 29 men and 11 women. Twenty-eight patients (70 per cent) reached uremia within one year. Ten patients progressed to uremia within four months (Table XIV). There were no special characteristics among these 10 patients with respect to age, sex or hypertension. Three patients were normotensive at the serum creatinine level of 5 mg/100 ml, 4 *patients* were hypertensive and 3 patients developed malignant hypertension during the progress of glomerulonephritis. Malignant hypertension was present in 6 of the remaining 30 patients.

The mean time for progress until terminal uremia was 10.1 months with a range of 0.7 to 36.8 months for the 40 patients. There seemed to be a difference between men and women, the mean duration being 11.6 months in 29 men and

Table XIV. *Distribution of 143 patients according to renal disease and the time from chronic azotemia to terminal uremia*

	<4 months	>4 <12 months	>12 <24 months	>24 months	Total no.
Pyelonephritis	10	19	14	9	52
Glomerulonephritis	10	18	9	3	40
Diabetes mellitus	10	11	3		24
Polycystic kidney disease		4	6	1	11
Nephrosclerosis	2	6			8
Amyloidosis	5	2	1		8
Total	37	60	33	13	143

5.9 months in 11 women. This difference was however not statistically significant. Compared to the rate of progress for pyelonephritis there was a tendency towards more rapid progress of glomerulonephritis ($0.05 < p < 0.1$).

Diabetes

The rate of progress was estimated in 24 patients with diabetic nephropathy 10 men and 14 women. Twenty-one (88 per cent) of 24 diabetic patients died from uremia within one year (Table XIV). Nine patients whose renal disease progressed to terminal uremia in less than four months were hypertensive at a serum creatinine level of 5 mg/100 ml. One woman (59 years old) was not hypertensive but reached terminal uremia in less than three months. Another 3 non-hypertensive patients died from uremia after about seven months. There was no correlation between the duration of the diabetes and the rate of progress of the renal insufficiency. The mean duration of the diabetes among the 24 patients was 19 years. Seven patients had their diabetes diagnosed before the age of 16. Six of them progressed to uremia within six months after having exceeded a serum creatinine level of 5 mg/100 ml. The remaining patient died after 13 months.

The mean time for progress to terminal uremia was 6.0 months with a range of 0.5 to 14.2 months. The rate of progress of diabetic nephropathy was significantly more rapid ($p < 0.05$) than for glomerulonephritis or non-obstructive pyelonephritis.

Polycystic kidney disease

The time to terminal uremia could be determined for 11 patients, 3 men and 8 women with chronic azotemia due to polycystic kidney disease. Hypertension was seen in 8 patients but the material was to small for any correlations of age or sex to the rate of progress of renal insufficiency. The mean time from a serum creatinine level of 5 mg/100 ml to terminal uremia was 18.1 months with a range of 5.6 months to 40.8 months. The rate of progress for glomerulonephritis and diabetic nephropathy was significantly more rapid ($p < 0.05$) than for polycystic kidney disease. There was no difference between the rate of progress of pyelonephritis and polycystic kidney disease.

Nephrosclerosis and amyloidosis

Eight patients among the 17 patients with nephrosclerosis fulfilled the criteria for estimation of the rate of progress to uremia. The mean time from the initial chronic azotemia until terminal uremia was 5.7 months with a range of 2.0 months to 9.0 months. Eight of 17 patients with amyloidosis fulfilled the criteria for estimation of the rate of progress to uremia. The mean time was 5.3 months with a range of 0.2 months to 19.6 months from chronic azotemia until terminal uremia.

The number of patients in these two groups was too small to represent the whole group of patients with these diseases and a comparison with the rate of progress of other diseases was therefore considered to be misleading.

Terminal uremia

In order to determine the total mortality in uremia all deaths from uremia among the population of Gothenburg between the ages of 16 and 75 years and all patients receiving active treatment with dialysis or kidney transplantation were included which made a total of 302 patients. These were 51 of the 58 patients with chronic azotemia previously described with azotemia developing before 1966 of whom 5 died from causes other than terminal renal failure and 2 patients survived the period, and 251 of the 369 patients with azotemia developing during 1966–1971 (Table XV). The seven patients with azotemia developed before 1966 are accounted for previously. Details of the included 51 patients are shown in Table XVI.

One hundred and eighteen patients not included of the 369 patients with azotemia developing after 1966 are accounted for in Table XVII. These 118 patients were not included because 51 survived the period and 67 died from causes other than uremia, as is apparent from Table XVIII. Eighty patients received active treatment of the 302 patients with terminal uremia and 222 died from uremia without receiving active treatment.

In all but ?7 (9 per cent) of the 302 patients, 154 men and 148 women, the diagnosis was investigated histopathologically. The age and sex distribution for the 302 patients is shown in Fig 8. Twenty four per cent of the patients were older than 65 years. The renal diseases causing uremia

Table XV *Patients aged 16–75 years with terminal uremia during 1966–1971*

	No.	No of non-uremic deaths	Patients alive after 1971	No. of patients with terminal uremia	
Azotemia due to renal disease					
Azotemia developing before 1966	58	5	2	51	
Azotemia developing 1966–1971	369	67	51	251	
Total no				302	
Patients dying from uremia 1966–1971					222
Patients actively treated 1966–1971					80
Total no					30?

Table XVI *Distribution according to disease in 51 patients with azotemia developing before 1966 and reaching terminal uremia during 1966–1971*

	1966	1967	1968	1969	1970	1971	Total no
Pyelonephritis	16	8	2	3	1		30
Glomerulonephritis	5			1			6
Polycystic kidney disease	1	2					3
Diabetes	2	?					4
Nephrosclerosis	3						3
Amyloidosis	1	1					2
Systemic lupus erythematosus		2					2
Other diseases	1						1
Total no.	?9	15	2	4	1		51

Table XVII *Distribution per year of diseases among patients aged 16–75 years with azotemia developing during 1966–1971 and who did not reach the state of terminal uremia during the period.*

	1966	1967	1968	1969	1970	1971	Total
Pyelonephritis	9	9	8	7	9	11	53
Glomerulonephritis		4	3	1	3	9	20
Diabetes	4	1	2	1	2	5	15
Nephrosclerosis	1	1	4	4	1		11
Polycystic kidney disease					2	2	4
Amyloidosis		1				1	2
Systemic lupus erythematosus	1					1	2
Other diseases	1	3	3	2	2		11
Total	16	19	20	15	19	29	118

Table XVIII *Causes of death among 67 azotemic patients dying from causes other than uremia during 1966–1971*

	Cardio-vascular	Cerebro-vascular	Broncho-pneumonia	Other causes	Total no.
Pyelonephritis	19	4	2	3	28
Glomerulonephritis	5	2		2	9
Diabetes	7	2	1	2	12
Nephrosclerosis	5	2	3		10
Amyloidosis	1				1
Systemic lupus erythematosus	1				1
Other diseases	2			4	6
Total no	40	10	6	11	67

Table XIX. *Distribution of diseases for each year (1966 to 1971) among patients with terminal uremia.*

	1966	1967	1968	1969	1970	1971	Total
Pyelonephritis	4	23	28	15	17	14	121
Glomerulonephritis	14	4	7	11	17	10	63
Diabetes mellitus	4	13	6	6	4	5	38
Polycystic kidney disease	3	4	5	2	2	3	19
Nephrosclerosis	4	5	1	2	3	2	17
Amyloidosis			2	4	3	4	17
Systemic lupus erythematosus		3	1		1	1	6
Other diseases	4		1	6	4	4	21
Total no	55	56	51	46	51	43	307

are stated in Table XIX. Pyelonephritis, glomerulonephritis and diabetic nephropathy were the most common causes of uremia, constituting 74 per cent of the 302 cases. The age and sex distribution for the 222 patients included in these three groups is shown in Fig. 9 Pyelonephritic patients were most often aged between 56 and 65 years, glomerulonephritic patients most often between 46 and 55 years while patients with diabetic nephropathy had two age maxima.

Owing to a policy change in the kidney transplant programme a false date for terminal uremia was given for 4 patients. One patient with chronic pyelonephritis (52 years old) received a cadaver kidney transplant in 1970 which rejected and was removed after 13 days. His renal function was sufficiently good to allow conservative treatment for a further seven months before hemodialysis was necessary. Consequently the year of terminal uremia ought to be changed from 1970 to 1971 The same change from 1970 to 1971 might apply to 1 patient (51 years old) with slowly progressing glomerulonephritis as he was transplanted before uremia at a serum creatinine of 7.5 mg/100 ml. One patient (53 years old) with polycystic kidney disease was operated on at a serum creatinine of 6.8 mg/100 ml and the year of terminal uremia might be changed from 1969 to at least 1970 as her renal insufficiency was slowly progressing. The fourth patient (60 years old) was nephrectomized bilaterally due to renal pelvic carcinoma in 1970 at a serum creatinine of 5.5 mg/100 ml and was treated by hemodialysis. He had a phenacetin nephropathy Without this interruption in his renal insufficiency the year of terminal uremia ought to be 1971 instead of 1970 The influence of these 4 patients on the mortality in uremia during the period is given in Fig. 10

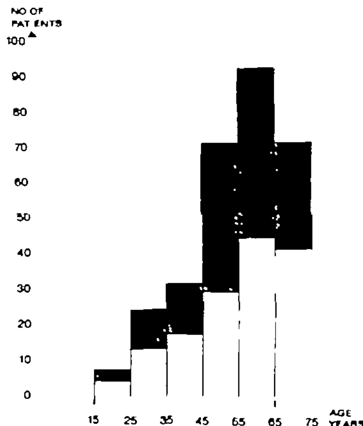


Fig. 8. Age and sex distribution among 302 patients with terminal uremia during 1966 to 1971 Unfilled areas represent men and shaded areas women.

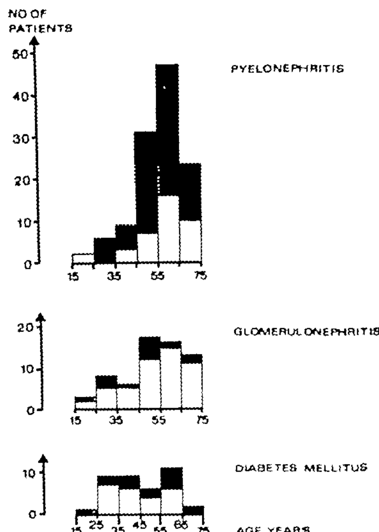


Fig 9 Age and sex distribution of patients with pyelonephritis, glomerulonephritis and diabetic nephropathy causing terminal uremia during 1966–1971 The unfilled areas refer to men and the shaded areas t[illegible] women.

INCIDENCE OF MORTALITY IN UREMIA

The distribution of 30[illegible] patients aged 16–75 years included in the calculations of mortality in uremia over the years investigated is seen in Table XIX as well as the distribution by renal disease

A maximum incidence was seen in 1967 with 56 patients and the minimum incidence was 43 in 1971 A decreasing tendency was found A comparison between the number of patients included in the mortality in uremia in 1966 1968 and 1969–1971 showed no significant change The mean total mortality in uremia was 150 cases per year and million inhabitants The [illegible] nations in mortality during the period [illegible] shown in Fig 10 The incidence of mortality [illegible] 170 cases per million inhabitants in 1966 and 126 cases per million inhabitants in 1971 The calculated mortality in uremia constituted 7.3 per cent of the causes of death among deceased citizens aged 16 75 years in Gothenburg.

The distribution of 230 patients in the age groups 16–65 years on the years investigated is demonstrated in Table XX. The table is corrected for the previously mentioned 4 patients in whom the natural course of renal disease was interrupted. The mean mortality in uremia for the period was 178 cases per year and million inhabitants. The variations between the years investigated is shown in Fig 11 The maximum incidence was 155 cases

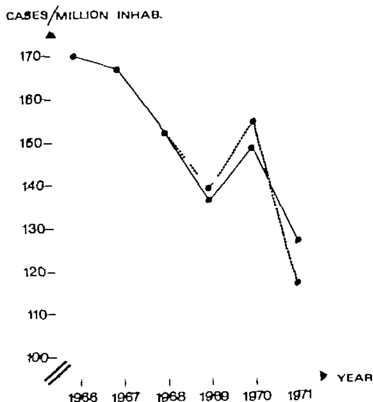


Fig. 10. Calculated mortality in uremia per year and population 16–75 years. The unbroken line represents mortality figures corrected for 4 transplanted patients and the dotted line indicates uncorrected mortality figures (see text).

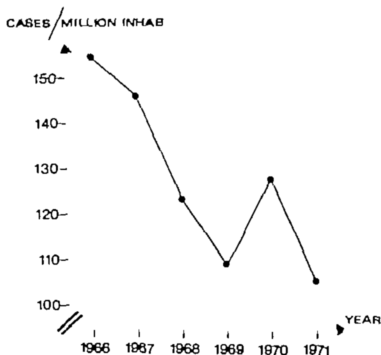


Fig. 11 Incidence of deaths from uremia per million inhabitants aged 16–65 years and year

Table XX. *Distribution of patients aged 16 to 65 years according to renal disease during 1966 to 1971*

	1966	1967	1968	1969	1970	1971	Total
Pyelonephritis	21	17	19	12	14	12	95
Glomerulonephritis	12	3	6	8	13	8	50
Diabetes mellitus	4	11	6	6	4	5	36
Polycystic kidney disease	2	4	4	2	1	3	16
Amyloidosis	1	2	1	3	3	2	12
Nephrosclerosis	3	2			1		6
Systemic lupus erythematosus		3	1		1	1	6
Other diseases	2	2		2	2	1	9
Total no	45	44	37	33	39	32	230

per million inhabitants in 1966 and the minimum 105 cases in 1971 No significant change was found when the mortality in uremia for 1966–1968 was compared to that for 1969–1971

Pyelonephritis

Pyelonephritis was the cause of uremia in 121 patients, 38 men and 83 women, in the age group 16–75 years. The pyelonephritic patients were divided into three sub-groups. Seven patients were included in the sub-group with obstructive pyelonephritis (I), 49 patients in the sub-group with non-obstructive pyelonephritis (II) and 65 patients in the sub-group with phenacetin nephropathy

The 7 patients with obstructive pyelonephritis were 5 men and 2 women. One woman (65 years old) was unilaterally nephrectomized because of hydronephrosis with infection 13 years before she died from uremia She had a cutaneous ureterostomy because of a malignant tumour in the urinary bladder one year before death She had recurrent urinary tract infections and a serum creatinine level of 4 mg/100 ml before removal of the tumour No signs of tumour tissue and no signs of papillary necrosis were found at necropsy The other woman (35 years old) had hydronephrosis hydroureter and ureter stenosis of [illegible]ongenital origin She also had a history of abuse [illegible]f phenazone [illegible]f long duration. No papillary necrosis was proven radiologically Three male patients ([illegible]0 60 and 63 years old) suffered from repeated urinary [illegible] and recurrent urinary tract in[illegible] The remaining 2 male patients (74 ye[illegible] d 43 years old) had hydronephrosis and recurrent [illegible]nary tract infections 1 patient because of hyperplasia of the prostata and the other because of reflux to one of the kidneys, calculus formation in the bladder and a suspected stricture of the urethra.

Among 49 patients (16 men and 33 women) in the sub-group of non-obstructive pyelonephritis (II) 12 patients (4 men and 8 women) had proven papillary necrosis either at necropsy (11 patients) or radiologically (1 patient) Seven of these 12 patients denied abuse of phenacetin-containing drugs. For the remaining 5 patients a history of phenacetin abuse was lacking. One of the 4 male patients (58 years old) had a malignant tumour of the urinary bladder diagnosed the same year as he died from uremia. Two female patients in sub-group II had been treated for cancer colli uteri (53 years old) and cancer mammae (61 years old) diagnosed four years and eight years respectively before their death from uremia. One male patient (75 years old) died from uremia during an acute deterioration caused by urinary tract infection in combination with dehydration.

The sub-group (III) with phenacetin nephropathy consisted of 65 patients, 17 men and 48 women In 4 patients in sub-group III no papillary necrosis was found at necropsy In another 4 patients no necropsy was performed and no signs of papillary necrosis were found upon radiological investigation All 8 patients had a long history of phenacetin abuse

Among the 65 patients in sub-group III 10 demonstrated malignant tumours. Renal pelvic carcinomas were found in 4 patients and malignant tumours of the urinary bladder in 2 patients. Two patients were found to have unilateral hyper

nephromas at necropsy One patient (75 years old) was treated for cancer of the prostata diagnosed six years before his death from uremia. One patient (58 years old) had been treated for cancer mammae 14 years before dying from uremia. None of the 4 latter patients were judged to have died from any complication from these tumours but from uremia due to phenacetin nephropathy

The number of patients dying from uremia because of pyelonephritis showed a decreasing tendency during the period (Table XIX). Twenty four patients were found in 1966 and 14 patients in 1971 When the number of patients dying from uremia during 1966–1968 was compared to the number of deaths from uremia in 1969–1971 a significant decrease in the number of patients was found ($p < 0.05$). When the first three years were compared to the last three years of the period there was a total decrease of 15 patients in the sub-group with non-obstructive pyelonephritis (II). This decreasing tendency was almost solely found among women, who decreased significantly in number from 23 to 10 between the two three year periods If the 1 probable phenacetin abusers in sub-group II are excluded the decrease in the number of women still persists – from 18 to 7 patients between the two three-year periods The numbe of men fell from 7 to 5 patients

The number of patients with phenacetin nephropathy (III) decreased by 15 from the first three year period to the last three-year period. This decrease was significant for women with a decrease from 34 to 14 between the two three-year periods When the 12 probable phenacetin abusers from sub-group II were included a significant decrease from 39 women to 17 was found. The number of men changed from 8 to 13 between the two three year periods. The proportion of men to women changed significantly from 1966–1968 to 1969–1971

When the patients in the sub-group with non-obstructive pyelonephritis (II) were distributed below and above an age limit of 55 years the number of patients aged 16–55 years was 8 out of 37 patients (Table XXI). The twelve probable phenacetin abusers have been transferred to the sub-group with phenacetin nephropathy (III).

When applying the same age limit to patients in sub-group III the number of patients in the age group 16–55 years was 37 out of 77 patients (Table XXI). A significant decrease from 22 women to 7 was seen when the number of women from the first three years was compared to the number in the last three years of the period investigated. A minor change from 17 to 10 women was noted in the age group 56–75 years. The mean mortality in uremia due to pyelonephritis was 60 cases per year and million inhabitants at ages 16–75 years. The variations during the years were 75 69 84 44 50 and 41 cases per year and million inhabitants from 1966 to 1971

Twenty-six (21 per cent) of the 121 patients with pyelonephritis were older than 65 years. The incidence of pyelonephritic patients in the age group 16–65 years showed a significant decrease when the numbers of the two three-year periods were compared ($p < 0.05$) (Table XX) with a maximum number of 21 patients in 1966 and a minimum number of 12 patients in 1971 A mean incidence of 53 cases per year and million inhabitants was found for the period investigated.

Table XXI *Distribution of patients with pyelonephritis in sub-groups II and III during the years 1966 1971 (see text)*

	Age years	1966	1967	1968	1969	1970	1971	Total no
II	16 55	2	3	1		1	1	8
	56–75	3	7	9	3	4	3	29
III	16–55	10	7	8	1	5	6	37
	56–75	7	5	10	8	6	4	40

The incidence decreased from 73 cases per million inhabitants in 1966 to 40 cases per million inhabitants in 1971 (Fig. 12) at ages 16–65 years.

Hypertension. Pyelonephritis as the cause of renal insufficiency was for 11 patients (9 per cent) of 121 patients not known until the serum creatinine level was above 5 mg/100 ml when the patient was first admitted to hospital. Fifty nine (54 per cent) of the 110 patients who were assessable with respect to hypertension at the serum creatinine level of 5 mg/100 ml were hypertensive. Two of the 7 patients in the obstructive sub-group (I) of pyelonephritis were hypertensive compared to 8 out of 14 men and 13 out of 28 women in the non-obstructive sub-group (II) and *12 out of 15 men and 24 out of 46 women in* the sub-group with phenacetin nephropathy (III).

Of the 12 probable phenacetin abusers with proven papillary necrosis, 9 were assessable with respect to hypertension. If these 9 are transferred from sub-group II to sub-group III, 15 out of 18 men (83 per cent) and 26 out of 52 women were hypertensive. In the pre-uremic state a further 16 patients were hypertensive. A total of 60 per cent of the patients with pyelonephritis had hypertension in the pre-uremic state. Five patients showed signs of malignant hypertension. Four of these patients (1 man and 3 women) were below 50 years of age and all were included in sub-group III. One of the 3 women had a renal artery stenosis when investigated radiologically. The fifth patient (56 years old) was found to have a renal artery stenosis on angiographic investigation. As there was no known phenacetin abuse and necropsy had not been performed she was referred to sub-group II.

Urinary tract infection. The record of urinary tract infection was incomplete in 12 patients in the non-obstructive sub-group (II) of pyelonephritis and in 7 patients in the sub-group with phenacetin nephropathy (III). *No history of urinary tract* infection and no pathological bacterial cultures were found for 2 patients in sub-group II. Both patients had proved papillary necrosis but no history of phenacetin abuse. Recurrent urinary tract infections were recorded for 62 per cent of the assessable 37 patients in sub-group II.

In sub-group III 5 of the 12 assessable men and 7 of the 46 assessable women had no history of urinary tract infection. Recurrent urinary tract

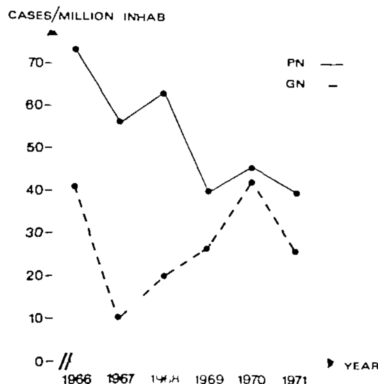


Fig. 12. Incidence of terminal uremia in pyelonephritis and glomerulonephritis per year and population 16–65 years in the city of Gothenburg.

infections were present in 41 per cent of the assessable 58 patients in sub-group III.

Malignancy In 4 of 65 patients with phenacetin nephropathy renal pelvic malignancy was found and there were also 2 cases of malignant tumours of the urinary bladder Only 1 patient out of 49 patients with non-obstructive pyelonephritis (II) had a malignant tumour of the bladder This patient had proven papillary necrosis but no history of phenacetin abuse There were no other known malignant tumours of the urinary tract in sub-group II.

Glomerulonephritis

Glomerulonehpritis was the cause of uremia in 63 patients, 49 men and 14 women at ages 16–75 years. Histopathological verification of the clinical diagnosis was lacking for 7 patients. Three men (53 54 and 67 years old) among these 7 patients had a more than ten-year history of proteinuria. One of them had a clinical diagnosis of glomerulonephritis seven years before he died from uremia. The other 2 had erythrocyturia and no recorded urinary tract infection. One of them developed malignant hypertension one year before dying from uremia. Two other patients (38 and 63 years old) were treated with immunosuppressive drugs due to clinical and laboratory findings suggestive of glomerulonephritis One man (62 years old) had erythrocyturia and proteinuria for many years before dying f om uremia. The laboratory findings for the remaining patient (51 years old) were equally suggestive of nephrosclerosis as of glomerulonephritis. The reduced renal size on enal angiographic investigation together with a normal blood pressure five years before his death from uremia favoured glomerulonephritis.

Two men with histopathologicaily verified glomerulonephritis (67 and 55 years old) also showed histopathological interstitial changes and signs of acute pyelonephritis respectively One female patient (67 years old) had a history of abuse of phenacetin-containing drugs for more than ten years but only for a few days each month. When re-evaluated at the department of pathology the microscopic examination showed in some areas papillary sclerosis and interstitial changes. The impression of mainly glomerular lesions was however not altered compared to the diagnosis of glomerulonephritis before re-evaluation.

The number of patients with glomerulonephritis as the cause of uremia changed irregularly with a maximum of 17 patients in 1970 and a minimum of 4 patients in 1967 (Table XIX). When the first three-year period was compared with the last three years of the investigation there was a change in number from 25 patients to 38 patients. This did not, however represent a significant change This increasing tendency was due solely to an increase among men from 18 to 31 when the two three-year periods were compared. The mean value for the incidence of glomerulonephritis as the cause of death in uremia was 31 cases per year and million inhabitants for the six-year period.

The 50 patients with glomerulonephritis in the age group 16–65 years as the cause of death in uremia distributed over the years 1966 to 1971 are shown in Table XX. A maximum incidence of 13 patients was found in 1970 and a minimum incidence of 3 patients in 1967 No significant change was found when the first three years were compared to the last three years. The number of male patients changed from 14 to 24 between the two three-year periods. A maximum incidence of 43 cases per million inhabitants was found in 1970 and a minimum incidence of 10 cases per million inhabitants in 1967 (Fig. 12). The mean incidence for the period was 28 cases per year and million inhabitants.

Hypertension. Sixteen (25 per cent) of 63 patients with glomerulonephritis were diagnosed at a serum creatinine level above 5 mg/100 ml. Thirty five (74 per cent) of the assessable 47 patients at ages 16–75 years with glomerulonephritis had hypertension at a serum creatinine level of 5 mg/100 ml. Another 2 male patients became hypertensive in the pre-uremic state. A total of 41 out of 49 men and 10 of 14 women had hypertension in the pre-uremic state

Criteria of malignant hypertension were found in 10 patients (8 men and 2 women). Eight of these glomerulonephritic patients had their renal disease diagnosed at a moderately decreased renal function Two male patients were admitted to hospital for the first time with a serum creatinine level above 5 mg/100 ml and with malignant hypertension. All but 2 of these 10 patients passed an investigation of renal angiography without showing any signs of renal artery stenosis In the age group 16–65 years 84 per cent of the 50

patients were hypertensive in the pre-uremic state. Hypertension was found in 34 of the 38 men and in 8 of the 12 women.

Diabetes

Diabetic nephropathy was the cause of uremia in 38 patients at ages 16–75 years, 23 men and 15 women. Only 2 patients were older than 65 years. In 34 of the 38 patients histopathological verification of the diagnosis was available. One (45 years old) of the remaining 4 patients was known to have had hypertension, diabetic retinopathy and proteinuria for many years. The duration of his diabetic disease was 28 years. One patient (48 years old) was described previously. The 2 remaining women (31 and 62 years old) had a history of diabetes for 17 and 12 years respectively. Both had a clinically verified nephropathy for about five years.

The incidence of diabetic nephropathy causing terminal uremia varied between 4 and 6 patients a year but with a maximum of 13 patients in 1967 (Table XIX). No significant change in the number of diabetic patients dying from uremia was seen when the first three years of the investigation were compared to the last three years. The mean incidence of diabetic nephropathy as the cause of terminal uremia was found to be 19 cases per year and million inhabitants for the period investigated at ages 16–75 years. Applying an upper age limit of 65 years meant a mean incidence of 20 cases per year and million inhabitants.

Hypertension. Twenty-eight (74 per cent) out of 38 patients were hypertensive at a serum creatinine level of 5 mg/100 ml. In the pre-uremic state 87 per cent of the patients had hypertension. Two men out of 23 and 3 women out of 15 had no hypertension in the pre-uremic state.

Polycystic kidney disease

Nineteen patients had polycystic kidney disease: 7 men and 12 women. All were diagnosed clinically and at necropsy. The incidence of polycystic kidney disease leading to uremia varied between 2 and 5 patients a year (Table XIX). This meant a mean incidence of 9 cases per year and million inhabitants. Four of the 7 men and 9 of the 12 women were younger than 56 years.

Hypertension. Two patients with polycystic kidney disease were not known at hospital until the serum creatinine of the patients was above 5 mg/100 ml. Four of the remaining 6 men and 8 of the 11 women had hypertension at the serum creatinine level of 5 mg/100 ml. No patient developed malignant hypertension. In the pre-uremic state 84 per cent of the patients had hypertension. In the age group 16–65 years 14 of the 16 patients had hypertension in the pre-uremic state.

Nephrosclerosis

Among the 17 patients at ages 16–75 years (10 men and 7 women) with nephrosclerosis as the cause of uremia 1 patient (70 years old, described above) had no histopathological diagnosis to verify the clinical and laboratory findings. In 3 out of 17 patients azotemia developed before 1966 and all died from uremia in 1966 at the ages of 62, 65 and 70 years respectively. The last patient suffered from rheumatoid arthritis for the last nine years before dying but was found to have nephrosclerotic kidneys reduced in size at necropsy. She also had myocardial infarction at necropsy and the cause of her death was therefore judged to be a combination of uremia and myocardial infarction. Only 6 of the 17 patients were younger than 66 years. When the period 1966 to 1968 was compared with the period from 1969 to 1971 a change from 10 patients to 7 patients with nephrosclerosis at ages 16–75 years was found and from 5 to 1 for ages 16–65 years (Table XIX and XX). A mean incidence of 8 cases per year and million inhabitants was found in the age group 16–75 years.

Hypertension. Two patients were admitted to hospital with previously unknown renal insufficiency and with a serum creatinine level above 5 mg/100 ml. All the other 15 patients were hypertensive at a serum creatinine level of 5 mg/100 ml. Two patients had established signs of malignant hypertension during the course of the disease. Another 5 patients had severe hypertension with ophtalmoscopic findings corresponding to stage III according to the classification of Keith-Wagener-Barker. For 5 patients there was no record of examinations of the eyegrounds and 1 patient could not be examined owing to bilateral cataracts. The remaining 2 patients had milder changes at ophthalmoscopic examination.

Amyloidosis

Amyloidosis causing uremia was in all 17 patients in the age group 16–75 years secondary to a chronic disease. Four patients had a history of tuberculosis – pulmonary in 2, arthritic in 1 and 1 patient (63 years old) had a history of pulmonary tuberculosis more than 15 years before the onset of rheumatoid arthritis. Her tuberculosis was probably of no importance with regard to the appearance of amyloidosis compared to 25 years of polyarthritis complicated by asthmatic bronchitis. One male patient (44 years old) had a history of recurrent respiratory infections including pneumonia and bronchiectasies. Another man (62 years old) had a long history of chronic bronchitis and bronchiectasies visible on radiological examination. Two male patients had ankylosing spondylitis. The remaining 8 patients suffered from rheumatoid arthritis of long duration before succumbing in uremia.

The incidence of amyloidosis changed from 6 to 11 cases between the two three-year periods at ages 16 to 75 years (Table XIX). The mean incidence for amyloidosis as the cause of uremia for the six-year period was 8 cases per year and million inhabitants in the age group 16–75 years. When the number of patients in the age group 16–65 years for the two three-year periods was compared a change from 4 to 8 patients was observed (Table XX).

Systemic lupus erythematosus

A total of 6 patients, 2 men and 4 women with systemic lupus erythematosus had a renal involvement leading to uremia (Table XIX and XX). The diagnosis was histopathologically verified in all patients. Only 1 patient (female) was older than 45 years.

Other diseases

Twenty-one patients at ages 16–75 years with "other diseases" causing uremia included 6 patients with myeloma. One patient (described above) had a clinical diagnosis of myeloma but with a complex picture of the kidney at histopathological examination. Six patients were described above. Three male patients (56, 67 and 7? years old) had malignant tumours of the urinary bladder with urinary tract infections. The remaining 3 male patients (69, 67 and 73 years old) suffered from hypernephroma with metastases, nephropathia urica and leukemia myeloica respectively. Two women (60 and 71 years old) had nephropathia NUD and malignant tumour of the urinary bladder respectively. The number of patients was evenly distributed over the period investigated (Table XIX). Twelve patients were older than 65 years.

Miscellaneous observations. Sixteen per cent of the 121 pyelonephritic patients with terminal uremia had a history of duodenal or gastric ulcer, melena or gastrectomy due to ulcer. Fourteen out of these 19 patients had papillary necrosis but only 10 patients admitted phenacetin abuse. The remaining 5 patients without papillary necrosis were included in the sub-group with non obstructive pyelonephritis. A history of tuberculosis, mainly of pulmonary origin, was recorded in 8 per cent of 121 patients. Seven per cent of the patients were operated on for cholelithiasis.

Ten out of the 63 patients with glomerulonephritis causing terminal uremia had a history of melena or gastrectomy (16 per cent). Three patients had a history of pulmonary tuberculosis. Four patients had a history of rheumatic joint disease and all 4 had been extensively examined clinically and microscopically for signs of amyloidosis but no amyloidosis was found. One (65 years old) of these 4 patients also had a history of psoriasis. His terminal renal failure developed within one month. The kidney was characterized by petechiae macroscopically, slightly enlarged, pale and the microscopic picture favoured an acute glomerulonephritis with proliferation of the capsular epithelium and focal bleedings. About 10 per cent of the patients were operated on because of cholelithiasis.

Only two of the 3? patients with diabetic nephropathy had had gastroduodenal ulcers. Eleven per cent of the patients had undergone cholecystectomies. Being a generalized disease, the diabetic lesions of retinopathy, neuropathy and peripheral infection and ischemia as a sign of microangiopathy were present with varying degree of severity in the records of all patients.

patients with phenacetin nephropathy had no record of signs of urinary tract infection.

The importance of urinary tract infection for the rate of progress of renal failure in a patient who admits a low consumption of phenacetin-containing drugs is difficult to evaluate. In the present investigation phenacetin nephropathy with or without urinary tract infection was the most common cause of uremia.

Apart from discontinuing phenacetin abuse (45 64) long term antibiotic treatment (94 16) and intense antihypertensive treatment (68) might be factors of value to reduce the incidence of renal failure due to pyelonephritis. The fall in incidence observed for pyelonephritis in the present investigation indicates that patients with pyelonephritis might be protected from terminal uremia if pyelonephritis is early detected and adequately treated.

Glomerulonephritis. Hood et al. (32) found a small change in the number of cases with chronic glomerulonephritis from 31 to 28 and to 27 cases per year and million inhabitants when three different periods between 1950 and 1965 were compared. The present investigation gave a mean value of 25 cases per year and million inhabitants at comparable ages. The decreasing trend, albeit small, found by Hood (32) seemed to continue during 1966–1971 The yearly variations in the number of patients with glomerulonephritis in the incidence of mortality in uremia might reflect epidemiological changes but the period investigated was too short to allow further conclusions on this trend. Adequate treatment to reduce terminal uremia from glomerulonephritis is lacking at present (54).

Diabetes. A mortality incidence in uremia of 19 cases per year and million inhabitants was found for diabetic nephropathy at ages 16–75 years. Among patients with onset of diabetes before 15 years of age the nephropathy seemed to increase in importance as a cause of death (52). Six out of nine deaths were due to renal insufficiency in a study of 31 patients with diabetic nephropathy (91). Neither age of onset of diabetes nor its duration could be related to the outcome of the diabetic nephropathy Renal involvment increased with increasing duration of diabetes (1.). In a selected series of patients half of the patients with renal involvment succumbed in renal failure (51). Generally the main cause of death among diabetic patients was coronary heart disease (41 29).

Polycystic kidney disease. The incidence of mortality in uremia from polycystic kidney disease was 9 cases per year and million inhabitants at ages 16–75 years. Polycystic kidney disease was the cause of uremia in seven per cent of uremic patients at ages 16–65 years. This frequency corresponded to that of other reports (66 32).

Amyloidosis. The previously observed decreasing tendency (32) of amyloidosis as a cause of death from uremia could not be verified in the present study from the same geographical area. Using comparable age groups the present investigation revealed 9 cases per year and million inhabitants compared to 6 cases and 1 case per year and million inhabitants in the early fifties and early sixties respectively (32). In the present study the incidence was 8 cases per year and million inhabitants at ages 16–75 years. Applying an upper age limit of 65 years, it was found that amyloidosis increased compared to nephrosclerosis, systemic lupus erythematosus and the group "other diseases" At ages 16–65 years more than 5 per cent of the total mortality in uremia was due to amyloidosis. The most common cause of amyloidosis was rheumatoid arthritis. This was not unexpected as about 25 per cent of patients with rheumatoid arthritis are found to have amyloidosis at necropsy (3). This was also in accordance with the findings of deWardener (89) but in contrast to those of Kennedy (39) who found tuberculosis to be the most common cause of renal amyloidosis. A total frequency of 6 7 per cent of amyloidosis was seen in a serially investigated group of patients with renal diseases (82)

Nephrosclerosis was the cause of uremia in 6 per cent of the patients in the age group 16–75 years and 3 per cent of the patients at ages 16–65 years. This relatively unimportant cause of uremia might decrease further as the antihypertensive therapy available today prevents the development of renal failure At least half of the patients at ages 16–75 years had hypertension corresponding to stage III and IV on the Keith-Wagener Barker scale The frequency of severe forms of hypertension might even be underestimated in the present study as in 1/3 of the patients no ophthalmoscopic examination was performed. A change in the pattern of the causes of death from hypertension has already

started to occur (33 68, 4), with a reduction in the number of deaths from uremia but an increase in the number of deaths from myocardial infarction. The over-all prognosis for patients with malignant hypertension has improved markedly

Systemic lupus erythematosus and "other diseases S. L. E. was the cause of uremia in two per cent of the patients in the age group 16–75 years. The incidence or uremic deaths due to S. L. E. was 3 cases per year and million inhabitants in these age groups. In a series of patients with S. L. E. in an adjacent population area the incidence of definite S. L. E. was 28 cases per year and million inhabitants in 1964–1971 This was an increase compared to earlier investigations possibly due to improved diagnosis but possibly to an absolute increase. About one-third of the patients died from uremia (74) which is a higher figure than in the present study

The diseases included in the group "other diseases" constituted together 7 per cent of the causes of uremia at ages 16–75 years. Applying an upper age limit of 65 years reduced the importance of this group as a cause of uremia to 4 per cent.

Hypertension was registered in 54 per cent of pyelonephritic patients with azotemia assessable with respect to hypertension. In the pre-uremic state 60 per cent of the patients had hypertension. In studies reporting decreased renal function corresponding to more than 2.5 mg/100 ml in serum creatinine (5) and more than 3.0 mg/100 ml (48) 56 per cent of the patients with papillary necrosis and 70 per cent of the patients with pyelonephritis respectively were hypertensive In both reports an increased percentage of hypertensive patients with decreased renal function was found. The frequency of hypertension in patients with pyelonephritis varied according to ages studied, stage of renal insufficiency and selection criteria (34 27 48 20, 57 15). Fifty per cent of the patients included in the present sub-group with non-obstructive pyelonephritis and 59 per cent of the patients with phenacetin nephropathy were hypertensive at a serum creatinine level of 5 mg/100 ml. The proportion of hypertensive patients in these sub-groups differed from a previous report (5) where 63 per cent of the patients with non-obstructive pyelonephritis and 56 per cent of the patients with papillary necrosis were hypertensive at a serum-creatinine level above 2.5 mg/100 ml In a follow up study (10) of patients with non-obstructive pyelonephritis including phenacetin nephropathy there was an increase in hypertensive patients from 37 per cent initially to 65 per cent at the end of the follow-up period Twenty two per cent of the patients died during the follow-up study and only 4 of the deceased 4 patients were normotensive

Hypertension was present in 74 per cent of the patients with azotemia due to glomerulonephritis This figure increased to 81 per cent in the pre-uremic state The figures for hypertension in the present study were lower than those reported by Kurkus et al (48) who used a lower limit of blood pressure for defining hypertension

Previous reports of a discrepancy with more frequent occurrence of hypertension among the glomerulonephritic patients compared to the pyelonephritic patients (48) found support in this study This discrepancy in the prevalence of hypertension between glomerulonephritis and pyelonephritis was not found by Brod (15)

Seventy-four per cent of the patients with diabetic nephropathy in the present study showed hypertension at a serum creatinine level of 5 mg/100 ml In the pre-uremic state 87 per cent of the patients were hypertensive which is in accordance with other reports of advanced renal insufficiency when hypertension usually developed (91 51).

Hypertension, being the most common complication during the course of polycystic kidney disease (31) was present in 71 per cent of the azotemic patients. In the pre-uremic state this percentage increased to 84 per cent. This frequency of hypertension is in accordance with other reports (31).

Rate of progress of renal disease. In order to predict the number of patients that will become uremic the pattern of progress of various diseases is important. It was thus interesting to note that the rate of progress for diabetic nephropathy was significantly more rapid than for other renal diseases and that there was no correlation between the rate of progress of diabetic nephropathy and the duration of diabetes. The average rate of progress from a creatinine level of 5 mg/100 ml to terminal uremia was 6 months. The average rate of progress for glomerulonephritis was 10 months and for non-obstructive pyelonephritis 14 months.

The number of patients with glomerulonephritis with a serum creatinine of above 5 mg/100 ml when first admitted to hospital was significantly higher than for patients with pyelonephritis. This supported the statistical tendency of a more rapid rate of progress for glomerulonephritis than for non-obstructive pyelonephritis. This is further supported by the difference in age distribution for the incidence of azotemia and for the incidence of uremia (Fig. 3 and Fig. 9) as the patients with glomerulonephritis will succumb at an earlier age than the patients with pyelonephritis. The high incidence of phenacetin nephropathy also indicates a slow rate of progress for pyelonephritis as reported observations (36 93 44 43) that renal insufficiency progresses very slowly if the patient stops taking phenacetin-containing drugs. About 37 per cent of the azotemic patients with pyelonephritis had a progressive renal insufficiency after 1971 These 53 patients will however in about 80 per cent of the cases reach terminal uremia after this date

It is interesting that the frequency of hypertension at serum creatinine level 5 mg/100 ml was 83 per cent of the patients with diabetic nephropathy 73 per cent of the patients with glomerulonephritis and 60 per cent of the patients with non-obstructive pyelonephritis Malignant hypertension was present in 31 per cent of hypertensive patients with glomerulonephritis and in 6 per cent of hypertensive patients with non-obstructive pyelonephritis. This indicates that the presence of hypertension increased the rate of progress of renal disease

Terminal uremia. Before reaching the state of terminal uremia a progressive change in the milieu of all organs occurs, affecting the patients both physically and mentally The ultimate cause of death may therefore sometimes be disputable (14). "The final diagnosis of the cause of death is at best a good interpretation of events in the current state of the art" (17). Histopathological verification of the diagnosis from necropsies and renal biopsies was present in 91 per cent of the patients with terminal uremia.

Differences in methods and selection criteria used in different investigations on mortality from uremia make direct comparisons of the results difficult. Mortality data during the fifties and early sixties indicated a decreasing tendency in the number of patients succumbing from acute and chronic glomerulonephritis from different parts of the world (84 42 22) The number of patients dying from diseases of infectious origin showed, however an increasing tendency during the same period This might partly reflect a change in diagnostic habits since Kass (37) and others described the natural history prevalence and treatment of urinary tract infections. The decreasing tendency in the incidence of glomerulonephritis seems, however to be too great to be just coincidental (72) A total decrease in kidney diseases in the mortality statistics might also reflect a change in habits when issuing death certificates i.e if a chronic kidney disease was more often considered to be the secondary than the primary cause of death. The findings of Berg et al. of a decreasing tendency in the number of chronic nephritis and infections of the kidney at ages below 70 years (11) are interesting but they are based on mortality statistics as are also the above-cited investigations. The present investigation failed to demonstrate a significant decrease in the total mortality in uremia during the period 1966–1971

A comprehensive study in Scotland for 12 months (1968–1969) revealed a calculated mortality in uremia of 109 cases per million inhabitants at ages 0–64 years. A closer look into this study (66) showed that 40 per cent of the cases of uremia were related to diseases defined in the present study as pyelonephritis. Glomerulonephritis was the cause of uremia in 27 per cent of the cases at ages 15–64 years. The figures found in the present study for ages 16–65 years resulted in a mean value of 128 patients dying from uremia per year and million inhabitants in 1971 Pyelonephritis was the cause of uremia in 40 per cent of the patients and glomerulonephritis in 21 per cent. These figures were also in accordance with the prevalence figures in Scotland (55).

At ages 16–60 years the average mortality in uremia was 83 patients per year and million inhabitants during the six years of the present investigation. This was in accordance with the 83 cases per million inhabitants found in Switzerland in 1966 at ages 15–60 years (79).

Differences in diagnostic groups make comparisons difficult but the "erweiterte Nephritisgruppe" (22) corresponded on the whole with the

groups of glomerulonephritis and nephrosclerosis in the present study. The figure for mortality in uremia in Switzerland in 1966 from these causes was 39 cases per million inhabitants at ages 15–60 years corresponding to a mean value of 27 cases per year and million inhabitants in the present study at ages 16–60 years.

The prospective study of McGeown (56) in 1968–1970 showed lower figures for mortality in uremia. Terminal uremia corresponded to 49 cases per year and million inhabitants when neglecting the suitability for active treatment of uremia. The reliability of the study seemed to be low at the beginning of the study and the survey was therefore extended to the three years of the definitive investigation (23). The figures from this investigation for the estimation of the need for active treatment of uremia for suitable candidates were however similar to those found in the study of Pendreigh et al. (66) and Branch et al. (13).

The proportions of different renal diseases varied among different investigators but in most European reports (32, 13, 66, 2, 78, 84) pyelonephritis was the dominating cause of uremia. The main causes of terminal uremia in the present study were pyelonephritis, broadly defined (40 per cent) and glomerulonephritis (21 per cent).

It thus appears that pyelonephritis is still the dominating renal cause of terminal uremia. Its decreasing tendency during recent years has, however diminished its dominance and glomerulonephritis seems to be on the way to becoming the dominating renal cause of terminal uremia.

Other observations. The method of investigation used did not allow accurate calculation of the frequency of azotemia as many diseases with a transient renal insufficiency only were registered under the main heading without special registra- study the survival figure was 56 per cent of acute renal failure not including acute postrenal obstruction. This figure is in accordance with recently reported survival figures of acute renal failure (40). The survival figure was 5? per cent in the present study when all patients with extrarenal causes of renal insufficiency were included (Table IV).

The family history of all the patients with the most frequent renal diseases was taken but the reliability of information from relatives and the apparent discrepancy between this information and the record made this information of doubtful reliability. It was therefore not evaluated in the present study. The low reliability of family histories has been verified by other authors (6).

As the indications of an association between phenacetin and papillary necrosis increased a possible carcinogenic effect of its metabolites was reported (6, 9). In the present study malignant tumours of the renal pelvis and the bladder were found in seven per cent of patients with phenacetin nephropathy.

An association between analgesic abuse and peptic ulceration was also suggested with a frequency varying from 50 per cent or more (20, 93) to 20 per cent (44). A possible contributary role of salicylates in these studies cannot be excluded. Reports of salicylate consumption and peptic ulceration may indicate this (19). The composition of the abused phenacetin-containing drugs in Sweden only exceptionally contained salicylates (8). Thus salicylates cannot have been responsible for the gastroduodenal ulcerations found in 18 per cent of the patients with a history of phenacetin abuse. This is supported by the finding that 16 per cent of the patients with glomerulonephritis in the present study had melaena or had undergone gastrectomy, which is of the same magnitude as in the group of pyelonephritic patients.

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Cardiovascular Effects of Poisoning by Hypnotic and Tricyclic Antidepressant Drugs

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by

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CONTENTS

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I. Bevegård S. & Thorstrand, C. Central hemodynamics in severe poisoning by hypnotic drugs. Acta med. scand. 191 325 1972.

II. Hulting, J. & Thorstrand C. Hemodynamic effects of norepinephrine in severe hypnotic drug poisonings with arterial hypotension. Acta med. scand. 192 447 1972.

III. Thorstrand C. Cardiovascular effects of poisoning with tricyclic antidepressants. Acta med. scand. 195 505 1974

IV. Thorstrand, C. & Lindblad L.E. The effect of amitriptyline on forearm blood flow Scand. J. Clin. Lab. Invest. In print

V. Thorstrand, C., Bergström, J. & Castenfors, J. Cardiac effects of amitriptyline in rats. Scand. J. Clin. Lab. Invest. In print.

VI. Thorstrand, C. Clinical features in poisonings by tricyclic antidepressants with special reference to the ECG. Acta med. scand. In print.

These publications will be referred to by their Roman numerals, I–VI

ABBREVIATIONS AND SYMBOLS

$AVDO_2$	=	arteriovenous oxygen difference ml/l
AT	=	*amitriptyline*
BP	=	arterial blood pressure mm Hg
CO	=	cardiac output l/min
CVP	=	central venous pressure mm Hg
HD	=	hypnotic drug
HR	=	heart rate beats/min
i.v	=	intravenous
MAP	=	monophasic action potential
n	=	number of observations
NE	=	*norepinephrine*
p	=	probability
PA	=	pulmonary artery
$PaCO_2$	=	arterial carbon dioxide tension mm Hg
PaO_2	=	arterial oxygen tension mm Hg
r	=	coefficicient of correlation
SD	=	standard deviation
SEM	=	standard error of the mean
SV	=	stroke volum ml
SVR	=	*systemic vascular resistance units*
TCA	=	tricyclic antidepressant
U	=	arbitrary units

INTRODUCTION

Chloral hydrate first made by Liebig in 1832 is the oldest member of the hypnotic group (27) Its use waned when the first hypnotic barbiturate barbital was introduced into medicine by Fischer and von Mering in 1903 In the following years a large number of barbiturates were synthetized and were soon being widely used The mortality rate in poisonings was high reaching 20 per cent or more (16 49)

The results of treatment hardly changed until about 1950 when new principles, known as the Scandinavian method were introduced (16 17 49) This method was based upon centralized careful supervision support of vital functions and immediate treatment of complications In practice it involves an abundant supply of electrolyte solutions parenterally respiratory care and liberal indications for respiratory treatment, and some use of pressor amines. Central analeptics were excluded Subsequently the mortality rate in large unselected materials was reduced to about 1 per cent (16 17 49 67) However severe poisonings with profound hypothermia may still present therapeutic problems such as arterial hypotension possibly participating in a picture of clinical shock. Pulmonary congestion or pulmonary oedema, sometimes fulminant are also described during the course of the poisonings (18 45 57 60 67)

During the last two decades a number of psychotherapeutic drugs have been introduced in Sweden they have largely replaced the use of barbiturates. Tricyclic antidepressants (TCA) alone which are used in the treatment of depressive states, exceeded the total sale of barbiturate tab ets by 64 per cent in 1970 (46) The psychotherapeutic drugs also account for an increasing number of poisonings. In this connection TCA in particular have attracted a great deal of attention because of their side effects from the cardio-vascular system (20 25 53 59 71) which in massive overdosage may be serious or even fatal (20 22, 59) ECG changes such as distorted and broadened QRS complexes and arrhythmias, as well as hypotension, have been reported (23 25 35 55 59). The real nature of the ECG changes is still obscure

A direct depressive action on the myocardium is generally thought to explain most of the adverse TCA effects (6 29 53 63) As for the broadened QRS complex, changes in the myocardial cell membrane resulting in an altered distribution of intra- and extracellular electrolytes has been proposed (41) as has a disturbed autonomic balance with an adrenergic dominance (51) Poisonings by hypnotic and psychotherapeutic drugs nowadays make up a substantial proportion of emergency medical cases and the patients are generally comparatively young The complications usually concern the cardiovascular system but there seem to be no reports on hemodynamic studies with invasive methods in human poisonings Since TCA poisonings are accompanied by cardiovascular changes which remain to be clarified disagreement exists concerning the proper treatment. The same applies in some measure to hypnotic drug (HD) poisonings, which besides barbiturates include compounds like meprobamate glutethimide and metaqualone It is a clinical impression that the course of poisonings with these compounds closely resembles that of barbiturates (?7)

On the basis of cardiovascular investigations in patients with hypnotic and tricyclic antidepressant poisonings and animal experiments, the purpose of this paper is to report and analyse some data obtained with the aid of both invasive and noninvasive methods.

PART I
ELECTROCARDIOGRAPHIC STUDIES

MATERIALS AND METHODS

The influence of TCA and HD poisonings on ECG was studied in two retrospective human materials

In rats, different materials were used to study the TCA effect before and after beta-receptor blockade The effect on blood and heart muscle electrolytes was also investigated as was the effect on monophasic action potentials of the exposed heart.

The significance of differences between mean values was tested by Student s t-test.

A. Patients. *Tricyclic antidepressants.* 153 consecutive patients 64 men and 89 women with TCA poisoning admitted to the intensive care unit between January 1 1969 and May 6 1973 were included in a retrospective study Their mean age was 34 years. Amitriptyline predominated being the only drug taken in 41 cases and combined in another 71 cases with other drugs or alcohol The average dose of TCA ingested was 1048 mg but in 52 cases the dose could not be calculated for lack of information. The interval between tablet ingestion and admission to the ward averaged 7 hours. Coma was present in 57 per cent of the patients. Urine analysis for neuroleptics and blood tests for barbiturates by spectrophotometric methods were performed routinely in the central chemical laboratory As for TCA, there were only 17 patients in whom the plasma concentration (mean 1.9 μg/ml) was determined by gas chromatography

The ECG recordings used for the analysis of time intervals were generally performed less than one hour after admission. Both standard and chest leads were recorded Time intervals were measured in the lead where they were best defined usually CR_2 The QRS duration measured was referred to one of the following groups. < 0.10 0 10 0 11 0 12 and > 0 12 sec Paper speed was 50 mm/sec QRS times ≥ 0 11 sec were defined as prolonged. Heart rate above 90 beats/min. was defined as tachycardia. PQ and QT times were compared with normal values corrected for HR (32). A chest lead ECG was usually monitored during the comatose period However no detailed information was available about the frequency of various types of arrhythmias. The influence of beta-receptor blockade after i.v injection of 5 mg propranolol was studied in four patients.

Hypnotic drugs. The HD material consisted of 50 consecutive patients 23 men and 27 women, admitted to the intensive care unit in 1968 Their mean age was 39 years. Barbiturates were the only drug in 21 cases and mixed with other hypnotics or sedatives in 25 The latter were generally carbromal, glutethimide hydroxyzine meprobamate or methaqualone The mean barbiturate plasma concentration was 5.2 μg/ml. The interval between tablet ingestion and admission to the ward averaged 4 hours. 92 per cent of the patients were comatose on admission ECG recordings were performed in the same way as for the TCA material.

B **Animals.** Female Sprague Dawley rats weighing about 200 g were anesthetized with pentobarbital sodium (75–100 mg/kg intraperitoneally) The right jugular vein and the left common carotid artery were each cannulated with a polyethylene cannula (PE 60) Only amitriptyline was used in this study

Standard leads I–III were recorded continuously at low spreed but a paper speed of 250 mm/sec was used intermittantly to permit a detailed evaluation of the ECG

1 *Beta-adrenergic blockade.* Two rat materials 17 and 12 animals receiving 0.5 and 2 mg/kg AT i v respectively were divided in control groups and groups which had pretreatment with 0 1 mg propranolol ECG recordings were made during short repeated periods before and 1/2–2 min after the injections

2 *Plasma and heart muscle electrolytes.* In eight rats receiving AT 0.5– 1 mg/kg i v a blood sample of about 4–5 ml was quickly drawn from an intra-arterial cannula when obvious AT-induced ECG changes were present Simultaneously the heart was exposed and a piece of muscle was taken from the apex region The same procedure was performed in nine other rats without a preceeding AT injection Plasma electrolytes were analysed with a conventional autoanalyser The heart muscle content of Na^+ K^+ Mg^{++} and Cl^- was determined according to Bergstrom et al (8)

3 *Monophasic action potentials.* MAP was recorded from the epicardial surface of the exposed heart in seven rats A subclavian cannula (Stille) with an internal diameter of 1.6 mm and containing a copper electrode was used as the suction electrode catheter The proximal opening of the catheter was tightened around the electrode and a side opening was connected to an electrical air-suction pump with a suction pressure of about –400 mm Hg Unipolar recordings were obtained from the electrode the distal part of which ended 1 mm proximal to the tip of the catheter ECG standard lead I MAP and arterial blood pressure were recorded simultaneously on the electrocardiograph Using an electrical resistance the MAP was adjusted to a standardized suitable size for the recorder Only the MAP duration was evaluated which together with QRS was recorded before i v injection of AT 2 mg/kg and continuously for 30 sec. after the injection The recordings were then repeated every half minute until 5 min The MAP duration was measured 5 mm above the isoelectric line and expressed as a mean of 5 consecutive action potentials.

A. Patients. Tricyclic antidepressants. (VI) The initial mean heart rate was 97 beats/min. (range 50–145)· in 112 cases (73 per cent) HR was > 90 beats/min. The final value measured just before the patients left the ward was 82 beats/min. (range 50–125) The difference is significant ($p < 0.001$) In the HD material the HR was 82 and 80 beats/min during the corresponding initial and final recordings. This difference is not significant. The initial heart rate was significantly higher in the TCA material compared to the HD material but there was no difference between to two final values. There was sinus rhythm in all but four patients who demonstrated nodal or ventricular rhythm with varying frequency No other arrythmias were noted on the diagnostic ECG recordings except for occasional ventricular extrasystoles in a few cases.

Massive overdosage of TCA may result in very advanced ECG changes (VI fig. 3). The mean QRS time was 0 11 sec (range 0 06–0.30) In 65 cases (42 per cent) the QRS time was 0 11 sec. or more (VI, fig 4). The PQ time averaged 0 18 sec. (range 0 13–0.30) which is at the upper normal limit after allowing for the increased HR. In 43 cases (28 per cent) the PQ time was prolonged in relation to HR. The QT time which averaged 0.37 sec (range 0.29–0.65) was prolonged in 75 cases (49 per cent). The QT time correlated negatively with HR and was prolonged compared to a normal material (VI fig 5). There was no significant linear correlation between PQ and QRS times but a comparison of means showed that increased QRS times were accompanied by increased PQ times. The QRS time did not correlate significantly with HR (VI, tables II and III)

After beta blockade (III) there was no detectable change of the QRS configuration.

The ST segment varied somewhat in configuration but showed no consistent change The T waves were often somewhat broad and flattened in connection with marked QRS prolongations.

Hypnotic drugs. The HD poisonings (n=50) showed no change of PQ and QRS times. The values 0 16 and 0.08 sec respectively were all within the normal range except for one PQ time which was slightly prolonged. The differences between these ECG time intervals and those of the TCA poisonings were all highly ($p < 0.001$) significant. Only 3 of the HD cases had a QRS time of 0.10 sec (6 per cent) which was the highest value in this group The QT times were slightly prolonged in 24 per cent and the mean (0.38 sec.) was just below the upper normal limit. It did not differ significantly from the mean in the TCA material. Seven cases (14 per cent) were judged to be pathological in view of ventricular extrasystoles in two cases and ST depressions or T inversions in the remaining. Three of these cases also had QT prolongations.

B Animals. I.v injection of AT 0.5–2 mg/kg invariably led to a decreased heart rate the fall for the highest dose amounting to 19 per cent after 5 min. (V fig. 1). In rats with opened chest, HR decreased rapidly by 26 per cent after 1/2 min It then increased and after 5 min. was only 10 per cent below the pre-injection rate (V fig. 5).

Amitriptyline 0.5–2 mg/kg induced a prolongation of QRS and PQ duration which was maximal after 1/2 min. and for the highest dose amounted to 50 and 18 per cent respectively These values had decreased to 31 and 16 per cent respectively after 5 min. (V fig. 1

and table I) The electrical axis changed rapidly and the end result was a deviation to the right in about 80 per cent of the rats (V fig 2 and 3)

1 *Monophasic action potentials* The MAP was virtually unaffected initially after intravenous injection of AT 2 mg/kg while the QRS time rapidly lengthened reaching a maximal (+94 per cent) increase after 1 min. MAP duration then became slightly prolonged while the QRS duration fell back After five minutes the respective increases were 10 and 38 per cent (V fig 5) The QRS changes were statistically significant but not the changes in MAP

2 *Beta-adrenergic blockade* Propranolol in a dose of 0 1 mg caused a marked decrease in heart rate but only minor changes in blood pressure QRS and PQ duration Pretreatment with propranolol did not influence the effect of intravenous injection of AT on heart rate QRS or PQ duration (V table I fig 7)

3 *Plasma and heart muscle electrolytes* AT induced no detectable change in the plasma concentrations of Na^{+} K^{+} Ca^{++} and Cl^{-} and no detectable changes in the heart muscle contents of Na^{+} K^{+} Mg^{++} and Cl^{-} when obvious QRS prolongation was present

DISCUSSION

According to the clinical study of patients poisoned by TCA (VI), 64 per cent of the ECG recordings were classified as pathological, an increased QRS time being found in 42 per cent. The PQ and QT times were less markedly increased in relation to the upper normal time intervals for these parameters. The presence of ECG changes is in agreement with several reports that severe TCA poisonings are consistently accompanied by ECG changes, often with broadened and distorted QRS complexes (23 25 33 35 55 59). Noble and Matthew (50) reported only a few ECG changes with a broadened QRS complex but their material included mainly mild poisonings, the mean ingested dose being about half that in this material. 88 patients with TCA poisonings had QRS times within the normal range (≤ 0.10 sec) but several showed a decreased QRS time within a few days after drug intake. Thus, the true incidence of TCA-induced QRS prolongation is obviously higher than 42 per cent, which was the frequency of QRS ≥ 0.11 sec. Even marked QRS prolongations generally returned to normal within a week. In the animal experiments, too, a QRS prolongation was the most characteristic finding. Since it was not influenced by beta-adrenergic blockade, there is no support for the view that the ECG changes are caused by disturbed autonomic balance with adrenergic dominance (51). Nor can an increased vagal stimulation explain the QRS prolongation since bilateral vagotomy did not abolish the AT-induced ECG changes (V).

Furthermore, there were no significant electrolyte changes in blood plasma and heart muscle after an AT administration that elicited marked QRS prolongation. This contradicts the hypothesis of an imbalance between intra- and extracellular ions, due to changes in cell membrane permeability caused by AT, as a cause of the ECG changes (41), at least in an acute situation like the present study. On the other hand, membrane effects causing changes in the rate of cellular depolarisation cannot be excluded with the method used or with the aid of monophasic action potentials. The use of an intracellular technique revealed a slowed upstroke phase of the action potential (3). But as the MAP duration did not change, it is unlikely that the ECG changes were caused by heavy ionic imbalance in the myocardial cells. This view is also supported by the lack of a correlation between electrolytes and ECG in the clinical material (VI).

HD poisonings were not accompanied by changes in PQ or QRS times but there was a tendency towards QT prolongation. The lack of a significant difference between the QT times in the HD and TCA materials is probably explained mainly by the difference of 14 beats/min in heart rate. The HR might be somewhat decreased by barbiturates, which predominated in the HD material. Pentobarbital, selectively administered through the nutrient arteries of the sinus node and AV junction, has been shown to possess negative chronotropic and dromotropic effects (69). Sinus tachycardia, which has been attributed to an atropinelike effect of TCA (14 68), was another common finding. A direct depressive action of TCA has been proposed in severe cases (35 53 71). The bradycardia observed after intravenous administration in rats probably results from a cardiotoxic effect of high plasma levels of TCA. This is in accordance with the decrease in heart rate in the patients who died.

Arrhythmias other than sinus tachycardia are considered to be a common complication of TCA poisonings, possibly because arrhythmias are frequently found in cases with fatal outcome (20 25 55). Contrary to this but in agreement with another survey (50), no

arrhythmias were recorded apart from sinus tachycardia in the patient material and brady cardia in the animal material Four of the patients who died however displayed arrythmia of nodal or ventricular type TCA have been shown to possess quinidine-like effects on action potentials (3 57) and antiarrhythmic properties *in vivo* (44 66). Quinidine slows the rise but does not change the duration of the action potentials (70). Similar effects may be induced by TCA The same mechanism may also account for the broadening of the QRS complex As the speed of cellular depolarization is important for the propagation of impulses a decreased speed *e.g.* in the intracardiac conduction system will result in retarded impulse propagation The rapid pronounced changes in the electrical axis (V) like the PQ prolongation also support the concept of a marked effect mainly in the intraventricular conduction system

It is known that adrenergic strain may activate latent pacemaker cells (27) and may accordingly elicit arrhythmias of ventricular or nodal origin which are often accompanied by aberrant intraventricular conduction of impulses. The adrenergic influence forms part of the TCA actions which may be important in the genesis of the arrhythmias reported in severe poisonings. This could explain the beneficial effect of beta-receptor blocking agents on arrhythmias in TCA poisonings (24) In that study the authors, using practolol also reported a decrease of QRS time in six patients but none of them had sinus rhythm before the injection. Therefore the change in QRS ratio might have been caused by a nor malized direction of intraventricular propagation of the impulses after restoration of the sinus rhythm According to another report (51) TCA-induced QRS broadening was not influenced by beta-adrenergic blockade during sinus rhythm which is supported by the present study However except in the most severe cases, the potential risk of an adrenergic action facilitating the development of arrhythmias generally seems to be counteracted by the proposed quinidine-like properties of AT

PART II
HEMODYNAMIC STUDIES

MATERIALS AND METHODS

A. Patients. Invasive methods were used in the study of 32 patients with acute drug poisonings (I II III). Another 7 patients participated in a separate study of the peripheral blood flow (IV) Non-invasive studies of BP and HR were also performed in the retrospective HD and TCA (VI) materials already described

The methods and procedures used in the investigations have been described in detail in paper I–VI and only a brief summary is given below Unless otherwise stated BP stands for the systolic arterial blood pressure The statistical methods are the same as in part I.

Hypnotic drugs. 1 *Coma depth.* Ten patients with a mean age of 33 years were included in the study All presented a picture of severe poisoning with deep coma and a mean body temperature of 32.9 C at the time of the initial hemodynamic measurements. At least one further recording was performed when the patient had improved and demonstrated a superficial coma or was conscious. In one case only a single investigation was made when clinical signs of pulmonary oedema were present during the awake phase of the poisoning. Most poisonings were mixed barbiturates dominated in six of them and in four the dominant or only drug was glutethimide chlorpromazine chlorprothixene or propiomazine All patients but one had a period of respirator treatment.

The investigations included recordings of cardiac output, peripheral arterial blood pressure pulmonary artery and central venous BP heart rate and blood gases The pulmonary artery was catheterized by the floating catheter technique The position of the catheter tip was determined by observation of the pressure curve (10). Pressures were recorded on a direct-writing ultraviolet multichannel recorder (ABEM Ultralette) Mean pressures were obtained electronically CO was measured by the indicator dilution method, using indocyanine green The dye was injected as a bolus in the pulmonary artery or in 3 cases, a subclavian vein. Blood was drawn from the femoral artery through a Beckman Cardio-Densitometer at a flow rate of 20 ml/min., using a 50 ml glass syringe adapted to an electric pump Arterial and mixed venous blood was sampled simultaneously and arterial pH PaO_2 and $PaCO_2$ were measured by electrode technique.

2 *Norepinephrine.* In 12 other patients (mean age 53 years) with poisoning of about the same type and severity as those above but demonstrating hypotonia (mean arterial BP < 70 mm Hg), the hemodynamic measurements were performed before and during administration of norepinephrine (NE). NE was diluted in 0.9 per cent NaCl-solution and administered i v by means of a Harward automatic infusion pump Doses ranged from zero to 0.79 μg/kg/min Administration of NE was discontinued for at least 15 min. before basal hemodynamic measurements were carried out except in 4 patients who required a small dose of NE to keep the mean BP above 50 mm Hg. In order to obtain a steady state

each dose level was maintained for at least 15 min before undertaking measurements Parenteral dextrose-electrolyte solution was administered simultaneously in a different peripheral vein. Urinary output was adequate in all patients

3 *Retrospective study* Blood pressure was also recorded in the retrospective material of 50 patients described in part I The conventional cuff method was used and recordings were made every hour during the early phase of the poisoning the interval increasing as the patient improved Systolic blood pressure below 100 mm Hg was defined as hypotensive The data received during the initial stage were compared with those during a later period of 6 hours referred to as the final period when most patients were awake and without apparent signs of poisoning. The lowest BP values were chosen from each period

Tricyclic antidepressants. 1 *Central hemodynamics* This material consisted of 10 patients with a mean age of 32 years. All but one patient who had taken nortriptyline were amitriptyline poisonings Six of the cases were mixed poisonings but concentrations of drugs other than TCA were generally low Prolongation of the QRS time characteristic of TCA poisonings, was present in all cases. The maximal recorded QRS time averaged 0 16 sec and at the time of the first hemodynamic investigation 0 14 sec This investigation was performed while the patients were still comatose the body temperature being 35.3 C

A second investigation was performed on an average 37 hours later (body temp 37.3 C) when the patients were awake but not completely recovered There were still some ECG changes, the average QRS time being 0 10 sec Six patients received intermittent positive pressure ventilation Four patients were also investigated before and about 10 min after i.v injection of 5 mg propranolol both in the comatose and the awake states The TCA material was compared with 7 patients poisoned by HD (mean age 44 years) Five of them were included in the HD material described above Body temperature during the first and final recordings averaged 33.6 and 37 7°C respectively

2. *Peripheral effects* Forearm blood flow was recorded in 7 healthy male subjects with a mean age of 33 years (IV)

Four concentrations of AT were infused in the left brachial artery with the aid of a teflon catheter and an electric infusion pump Each concentration (0.05 0 10 0 15 and 0.20 mg/ml) was infused during a period of 5 min Calculated per kg body weight the doses ranged from an average of 0.38 to ? 7? µg/kg/min The interval between two consecutive infusions was about ?0 min Initially 0.9 per cent saline was infused to evaluate the effect of the injected volume *per se*. Blood flow in the left and right forearm was measured simultaneously using an air-filled plethysmograph (28). In order to diminish the influence of spontaneous fluctuations of the forearm blood flow the value during the infusion was compared to that during a control period just prior to this. The blood flows were recorded during the last 90 sec of the infusion Arterial blood pressure was recorded immediately before and after the infusion and the mean BP during the infusion was calculated as the mean of the two recordings. Local vascular resistance was calculated by dividing mean BP by forearm blood flow the result being expressed in arbitrary units. Heart rate was obtained from a continuous ECG recording.

In two subjects the effect of infusion of AT 0.20 mg/min was also studied after the sympathetic tone had been raised by applying subatmospheric pressure (—40 mm Hg) around the lower body (7?).

In a separate experiment 30 mg AT was injected intravenously and heart rate blood pressure and forearm blood flow were recorded every 5 min until 20 min

3 *Retrospective study* Blood pressure was recorded in the TCA material of 153 patients described in part I The same methods were used as described for the corresponding HD material

B **Animals.** The rat materials (V) presented in part I were also used for BP recordings, which were performed simultaneously with the other procedures already described The blood pressure was derived from a cannula inserted in one of the carotid arteries. Mean arterial blood pressure was determined from the pressure curve

A. Patients Hypnotic drugs. 1 *Coma depth.* During the stage of deep coma (characterized by hypothermia) cardiac output heart rate stroke volume and oxygen uptake were significantly lower than in the later phase of poisoning CO increased from 3 7 to 7 1 l/min. The rise amounted to an average of 0.85 l/min/°C increase in body temperature In most cases CO was within the expected normal range in relation to the calculated oxygen uptake Heart rate increased from an average of 72 to 97 beats/min and the stroke volume from 52 to 73 ml at the time of the final recording The calculated oxygen uptake increased by 27 ml/°C increase in body temperature

The systolic arterial blood pressure increased from 106 to an average of 116 mm Hg during the late stage of poisoning The mean BP was virtually unchanged (81 and 80 mm Hg resp) The pressures in the pulmonary artery were within the normal range in most cases but four patients had values close to the upper normal limit There was no significant change in mean PAP between the initial (14 mm Hg) and the final study (16 mm Hg) Central venous pressure was normal or somewhat low and did not change significantly during the course of the poisoning No correlation was found between changes in CVP and CO In six of the cases there were roentgenological signs of pulmonary congestion or oedema but there was no correlation to the pressure in the pulmonary artery The systemic vascular resistance was increased ($p < 0.001$) compared to values for healthy subjects of corresponding age (9) During the recovery from poisoning it decreased significantly ($p < 0.001$)

pH and blood gases varied depending on whether the patients had respirator treatment were breathing spontaneously or had extra oxygen added to the inspired air Mean pH was normal (7 42) Mean $PaCO_2$ was a low normal value (36 mm Hg) The lowest PaO_2 values in 3 patients (mean 41 lowest 28 mm Hg) indicated considerable physiological shunting of blood in the lungs. PaO_2 exceeded 100 mm Hg (max 190) on 6 occasions because of oxygen therapy

2 *Norepinephrine.* Following infusion of NE cardiac output rose from an average of 3.6 to 5 1 l/min with the highest dose rate This increase of 1.5 l/min was significant ($p < 0.001$) $AVDO_2$ was determined in nine patients it decreased significantly ($p < 0.01$) but since the relative increase in CO was more pronounced the calculated oxygen consumption rose slightly Excluding one patient with a pacemaker the heart rate increased from 81 to 87 beats/min but the difference was not significant Stroke volume increased by 16 ml from the initial value of 47 ml The change was significant ($p < 0.001$) BP increased in all patients the mean values being 60 and 89 mm Hg Mean PAP increased significantly ($p < 0.001$) from 15 to 19 mm Hg The corresponding values for diastolic PAP were 11 and 13 mm Hg ($p < 0.05$).

The SVR index was about the same in this material (31 U) as in a control group (30 U) of about the same mean age The index did not change significantly after infusion of NE Stroke work expressed in GmM (II) was markedly increased

pH and blood gases showed no systematic changes However base excess fell significantly ($p < 0.01$) following NE infusion and as in the previous material (I) PO was low in several cases despite oxygen supply

3 *Retrospective study* The initial and final heart rates (8 and 80 beats/min) did not differ significantly However when calculated as the mean lowest value during the respec

tive period the initial HR (74 beats/min) was lower ($p < 0.05$) Blood pressure increased significantly ($p < 0.001$) from 100 to 123 mm Hg.

Tricyclic antidepressants. 1 *Central hemodynamics.* CO averaged 6 4 l/min during coma and 6 7 l/min in the awake state The difference was not significant.

HR was elevated above normal limits for resting individuals being 97 and 105 beats/min. respectively The difference was not significant.

SV was 65 and 64 ml during the initial and final recordings respectively

$AVDO_2$ was normal or low during coma and increased from 33 to 43 ml/l after coma ($p < 0.05$) Oxygen uptake also increased ($p < 0.05$)

BP was normal in both investigations (123 and 128 mm Hg) and the slight increase of 4 per cent was not significant The mean BP did not change (91 mm Hg) neither did the mean PAP (15 mm Hg) and CVP (3 mm Hg)

pH and blood gases showed no significant changes.

In all four patients in whom beta-blockade was induced by injecting 5 mg propranolol HR and CO decreased and $AVDO_2$ increased both during and after coma In the case of HR the decrease during coma was 20 and after coma 13 beats/min. The decrease of CO was 2.6 and 1.2 l/min respectively The systemic BP was essentially unaffected

In the retrospective material (VI), 40 patients (26 per cent) were hypotensive (systolic BP < 100 mm Hg) The mean systolic BP after admission to the intensive care ward was 108 mm Hg and the value just before the patients left the ward was 118 mm Hg, which was significant ly higher ($p < 0.001$). The corresponding diastolic BP values were 74 and 75 mm Hg. Like the HR determined from the ECG, HR calculated as mean lowest value was significantly ($p < 0.001$) higher initially (91 beats/min.). The mean pH was 7.35 PaO_2 and $PaCO_2$ were 70.9 and 37.9 mm Hg respectively

2. *Comparisons with hypnotic drugs.* In HD patients in the invasive material, HR and CO were significantly lower ($p < 0.001$ and $p < 0.01$) and $AVDO_2$ and SVR higher ($p < 0.01$ and $p < 0.02$) during coma than in TCA patients. SV and the calculated oxygen uptake did not differ significantly between the two materials.

In the retrospective HD material the initial HR and systolic BP were significantly ($p < 0.001$) lower than in the TCA material. The final BP however was lower ($p < 0.05$) in the TCA material (117 mm Hg) than in the HD material (123 mm Hg) HR did not differ significantly (80 and 82 mm Hg respectively)

3 *Fatal poisoning.* Catheterization of a case with a fatal dibenzepine poisoning (included in paper VI) showed a reduction of CO to 1.3 l/min. CVP was 19 mm Hg. Intracardiac pacing did not improve the condition

4 *Peripheral effects.* Intra-arterial infusion of AT in the brachial artery caused a dose dependent increase of forearm blood flow which on the highest dose rate 2 7 µg/min/kg, amounted to 73 % The calculated local SVR decreased significantly during infusion at all four dose rates. The decrease was most pronounced when the initial local SVR was high. No systemic effects were noted from the intra-arterial or in one case the i.v., infusion.

B Animals. Injection of AT 0.5–2 mg/kg in rats induced a gradual drop in BP amounting to 33 per cent after 5 min. on the highest dose level. In rats with opened chest and artificial ventilation the fall was less pronounced 20 per cent after 5 min Pretreatment with propranolol did not influence the effect of i.v. injection of AT 0.5 mg/kg but the dose 2 mg/kg induced a BP decrease.

DISCUSSION

Hypnotic drugs. It is well known that the circulatory changes following induced hypothermia are closely related to the reduction in tissue metabolism (11 31) Like the present study clinical experience from other materials of drug poisonings (8 42 74) shows a close correlation between coma depth and body temperature The calculated oxygen uptake and cardiac output correlated linearly to body temperature Consequently a reduced tissue metabolism during drug-induced deep coma could explain the low CO found in the HD poisonings (I) The total body arteriovenous oxygen difference was essentially normal and relatively unchanged at the different body temperatures This agrees with earlier investigations of the effect of hypothermia (11) It implies that CO varies according to metabolic demands as reflected in oxygen consumption

The mean BP was virtually unchanged despite the reduction of CO during deep coma which indicates a reflexly increased SVR This agrees with data reported by Shubin and Weil (61). These authors also attributed the reduction in CO to a relative hypovolemia. In rabbits pentobarbital has been shown to cause a redistribution of blood from skeletal muscles to kidneys and intestines (1) The presence of a certain hypovolemia is in agreement with earlier findings in this clinic (43) However since the reduction in CO was proportionate to the reduction in oxygen uptake and there was no correlation between CVP and CO hypovolemia is not likely to be the sole or even a major cause of the reduced CO

In all cases the CVP was low or normal Thus decreased myocardial function as refelcted in elevated ventricular filling pressures, cannot be a major cause of the reduced CO On the other hand barbiturates are known to be general cellular depressants (27) The slightly increased diastolic pulmonary artery pressure in a few cases may be an indication of disturbed left ventricular function Besides a reduced metabolic demand the finding of initially reduced HR and systolic BP in the retrospective material of HD poisonings may indicate the presence of a depressive effect on the circulation This effect is probably complex

The hemodynamic pattern that accompanies severe poisoning by hypnotic drugs may be caused by one or more of the following factors 1) hypovolemia (61) 2) inadequate tone of capacitance vessels (?1) 3) reduced myocardial force (1? 19) 4) impaired regulation of resistance vessels (5). In the literature a clinical picture which includes marked arterial hypotension is often referred to as shock (17 61 62) It is a matter of definition whether this term is justified or not The essential disturbance in the syndrome of shock is generally considered to be insufficient peripheral blood flow and tissue hypoxia which is not necessarily present in cases with hypnotic drug poisonings accompanied by hypothermia and reduced metabolic demand

Compared with the first HD material (I) those treated with NE demonstrated a less pronounced ability to increase SVR This could be due to the higher mean age in the NE group In healthy subjects the alfa-receptor stimulating effect of NE predominates The finding in this study that the pressor effect of NE is mainly accompanied by an increase of CO indicates that the beta-receptor stimulating effect on the myocardium may be quite marked. The positive inotropic effect of NE is reflected by the increase in left ventricular stroke work. Also there was usually no reflex bradycardia This indicates that the poisoning disturbed the normal BP regulation via the arterial baroreceptors

The interindividual variations in response to NE were considerable but available infor mation regarding the effects of the drug body temperature CVP or blood gas disturbances did not provide any simple correlation to the variation in NE response

Tricyclic antidepressants. The finding of a hyperkinetic circulation in TCA poisonings compared with patients suffering from HD overdosage cannot be explained by the differ ence in body temperature or other differences between the two materials apart from the kind of drug ingested. In the present material amitriptyline was the predominant drug. However clinical findings as well as the principal mode of action speak in favour of similar clinical and hemodynamic effects of other derivatives within the TCA group (15 48 53)

The effect of beta-receptor blockade indicated that there was a relatively increased adrenergic drive during the comatose phase of the poisoning. Adrenergic effects of intravenous infusion of TCA in low doses have been demonstrated earlier (26 58 63). A primary peripheral vasodilative effect of the drug with a subsequent increase in CO could explain the hyperkinetic circulation in this material This assumption agrees with the finding of vasodilatation following intra-arterial infusion of AT (IV). The local plasma concentrations of TCA in that study were calculated to be of the same order as those found in patients poisoned by TCA (23 64).

The blood pressure did not change significantly in the invasive study in contrast to the results in the larger retrospective study Hypotension from TCA has been reported in ear lier studies and generally attention has focused on the depressive actions on the heart (34 53 55 71) Clinical signs of myocardial depression are obviously a dominant feature in very severe poisonings by TCA This is illustrated by the findings in one of the fatal cases. The results of the animal experiments, in which the injected doses must be regarded as high, point in the same direction.

Beta-receptor blockade has been reported to be *effective* in the treatment of severe cases of TCA-induced arrhythmias (24 54). The negative inotropic effect *per se* of the doses required for beta-receptor blockade is probably not important. However the danger of myocardial insufficiency must be born in mind since it is possible that a certain adrenergic drive is appropriate to counteract a direct toxic TCA effect on the heart

Evaluated from the diastolic pressure in the pulmonary artery there was no left ventricular insufficiency

PART III
CLINICAL CONSIDERATIONS

MATERIALS AND METHODS

The material of 153 TCA poisonings described in part I has been studied with respect to a number of clinical variables and especially their relation to the ECG

The following clinical variables were used in the statistical analysis. Ingested dose of TCA, age duration of coma and intensive care initial and final HR and BP minimal body temperature serum K^+ and Na^+ and PQ QRS and QT times. Statistical analyses were performed in two ways. First the above-mentioned clinical variables were compared with one another according to linear regression analysis. Secondly the material was divided into paired groups with respect to differences in ingested dose ECG time intervals the presence of coma or not etc The means of the different variables in these paired groups were compared according to the Mann-Whitney test (37), which allows for uneven distribution The calculations were made with the aid of an IBM 360/75 computer

Concerning other materials or methods used in this part the reader is referred to descriptions in preceding parts.

RESULTS

There were a number of significant positive correlations, such as those between dose and the variables PQ, QRS and QT time (VI, table III). Dose and initial BP correlated negatively. There was no significant correlation between dose and initial HR, in contrast to the positive correlation to final HR. There was no significant linear correlation between PQ and QRS times but increased mean values of QRS were accompanied by increased PQ times (VI, table II). The QRS time did not correlate significantly to HR, age or time of coma. There was no correlation between the duration of coma and time of care, though the means of each of these variables differed significantly between patient groups with QRS ≤ 0.10 and those with QRS ≥ 0.12 sec. Plasma concentrations of K^+ and Na^+ were not correlated to either QRS, QT or PQ times. Sex and age did not influence the variables studied except for initial BP, which was lower in women. Nor were there any statistical differences between patients who had ingested AT only and those with mixed poisonings, the latter group including a limited number of TCA poisonings other than AT. Patients with coma or pathological ECG showed increases for variables such as time of care, dose of ingested TCA and QRS time when compared to patients who were awake or lacked ECG changes. The same was true for patients who suffered from convulsions or had respirator treatment. The concentrations of TCA in blood and urine showed no detectable relationship to the dose ingested or the degree of ECG changes.

DISCUSSION

Since young people dominated in the material used for analysis of clinical variables the incidence of preexisting ECG changes was probably small. The most severely ill patients, *i.e.* those with convulsions, coma, respirator treatment or fatal outcome were characterized by marked and highly significant QRS prolongation. Also there was a significant correlation between dose intake and QRS time in cases where the approximate dose was known. Thus, in the absence of previous ECG changes, the degree of QRS prolongation is a measure of the poisoning's severity. The lack of significant correlations to the duration of coma and time of care may be explained by factors other than the TCA effect itself. However patients with a markedly prolonged QRS time had a significantly higher incidence of prolonged coma. The finding that increased doses were accompanied by increased final heart rates may indicate a residual anticholinergic effect of TCA. In the initial stage the patients were probably submitted to more complex autonomic and direct toxic influences.

In severe TCA poisonings the ECG pattern is rather uniform and the ECG may be useful for distinguishing this type of poisoning from other drug poisonings (4). The finding that initial QRS times $\leq$ 0.10 sec. decreased within a few days after drug intake illustrates the diagnostic value of repeated ECG recordings in patients with suspected TCA poisoning.

The QT times also correlated with parameters reflecting the severity of TCA poisoning but they could not be measured so exactly owing to the difficulty in delimiting the end of the T wave from the U or P waves. Furthermore the percentage increase in QT time was comparatively small and thus less obvious than for QRS. Similar reservations apply to the less frequent PQ prolongation.

Arrhythmias other than sinus tachycardia were few. It seems that a massive dose of 1.5–2 g or more is generally a prerequisite in acute poisonings for the development of serious arrhythmias in adults. In such a situation, as already suggested it is probable that an increased adrenergic strain plays a role. The importance of sympathetic activity in producing arrhythmias in the presence of TCA has recently been demonstrated in isolated perfused rabbit hearts (6 7). Beta-receptor blocking agents may then be of clinical value (6 7 24 54).

The inhibitor of cholinesterase physostigmine has also been reported to have favourable effects on TCA-induced cardiac arrhythmias (36 65) but the ability to counteract proposed anticholinergic neurological symptoms like coma and hallucinations seems to be the most striking effect of the drug. Adequate treatment of acidosis by sodium bicarbonate is reported to be effective against arrhythmias during the influence of TCA (13). In the French literature (23 25 35) the same treatment is recommended when marked QRS prolongation is present. In one of the fatal cases in the present study the QRS time was markedly reduced just before the fatal arrhythmia occurred. The same experience was reported in a case by Sedal *et al.* (59). This indicates that the QRS prolongation *per se* is not necessarily responsible for the fatal outcome.

As in an earlier study (23), ECG and dose of TCA showed no correlation to the blood concentration of TCA. This is probably explained by factors such as small materials uncertainty regarding the ingested dose insufficiently exact blood tests, vomiting and interindividual differences in pharmacokinetics such as rate of drug metabolism (2). Sensitive

RESULTS

There were a number of significant positive correlations such as those between dose and the variables PQ QRS and QT time (VI table III) Dose and initial BP correlated negatively There was no significant correlation between dose and initial HR in contrast to the positive correlation to final HR. There was no significant linear correlation between PQ and QRS times but increased mean values of QRS were accompanied by increased PQ times (VI table II) The QRS time did not correlate significantly to HR age or time of coma. There was no correlation between the duration of coma and time of care though the means of each of these variables differed significantly between patient groups with QRS $< 0\ 10$ and those with QRS $> 0\ 12$ sec Plasma concentrations of K^+ and Na^+ were not correlated to either QRS QT or PQ times Sex and age did not influence the variables studied except for initial BP which was lower in women. Nor were there any statistical differences between patients who had ingested AT only and those with mixed poisonings, the latter group including a limited number of TCA poisonings other than AT Patients with coma or pathological ECG showed increases for variables such as time of care dose of ingested TCA and QRS time when compared to patients who were awake or lacked ECG changes. The same was true for patients who suffered from convulsions or had respirator treatment. The concentrations of TCA in blood and urine showed no detectable relationship to the dose ingested or the degree of ECG changes.

SUMMARY AND CONCLUSIONS

Central hemodynamics in poisonings by hypnotic drugs (HD) and tricyclic antidepressants (TCA) have been studied in 22 and 10 patients respectively.

The peripheral effect of intra-arterial infusion of the TCA compound amitriptyline (AT) was studied in 7 cases. The effect of TCA on ECG and its relation to other clinical variables have been analysed in a retrospective study of 153 patients. The cardiovascular characteristics of HD and TCA poisonings have been compared. A material of 50 HD poisonings was used for retrospective comparisons.

Animal studies concerning cardiovascular effects of AT are also included.

HD poisonings

1. During deep coma, characterized by hypothermia significantly lower values were found for CO, HR, SV and oxygen uptake compared to a subsequent recovery phase. $AVDO_2$ was essentially normal and did not change significantly with the changes in body temperature. This implies that CO primarily varies according to metabolic demands. The average increase of CO was 0.85 l/min/°C increase in body temperature.
2. In general, HD poisonings were accompanied by a moderate decrease in systemic BP. It seems that old patients are more prone to severe arterial hypotension than young patients. SVR was increased during the phase of deep coma.
3. Infusion of norepinephrine in patients with a mean arterial BP of 70 mm Hg or less resulted in significant increases in BP, CO and SV. SVR did not change significantly in contrast to earlier findings concerning the effect of NE in cardiogenic, hemorrhagic and septic states of shock.

   The rise in BP was primarily due to the increase in CO. Though not significant, HR was increased in 73 per cent of the cases. This finding, accompanied by an elevated arterial blood pressure implies a disturbed function of the high pressure baroreceptor system.
4. Roentgenological signs of pulmonary congestion or oedema during the course of poisoning do not seem to be explained by increased left ventricular filling pressure as reflected in elevated pulmonary artery diastolic pressure. There may be an increased permeability of the pulmonary vascular bed.
5. The QT time was slightly prolonged in 24 per cent (n=50) and PQ in 2 per cent. The QRS time was normal in all cases (mean 0.8 sec.). Plasma Na^+ was slightly increased (mean 146.5 mE/l). Plasma K^+ was normal. Except for PaO_2 which was often reduced, there were no marked changes of pH or $PaCO_2$.

TCA poisonings

6. Apart from an increased HR (HR ≥ 90 beats/min) which was present in 73 per cent of a material (n=153) of TCA poisonings (73 per cent AT) the most characteristic change was a QRS prolongation seen in 42 per cent (QRS ≥ 0.11 sec.). The PQ time was prolonged in 28 per cent and the QT time in 49 per cent. Unlike the QT time the QRS time was not correlated to HR.

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Stockholm May 1975 *Curt Thorstrand*

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By Knut Rasmussen

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ERRATA

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Right-hand column, second paragraph fourth line 16" should be 15

p. 10 Left hand column second paragraph, first line "Ca_O should be "Ca_O

p 16—17 Th ranges of the following variables should read

Men	TLC	RV	$\frac{RV}{TLC}$ %	$\frac{FRC}{TLC}$ %	FVC	FEV %	
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Women	FEV
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p 42 Table III first line Pa_{CO} "24 should be 29"

p 61 Table VI VC 63 % pred. range should ead 31—76

From: Medical Department B Rikshospitalet, University Hospital, Oslo, Norway

Evaluation of Vectorcardiographic Criteria in Different Kinds of Right Ventricular Overload

by
Knut Rasmussen

OSLO 1975

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Contents

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1 Repeat variability of axial lead electrocardiograms. J Electrocardiol. 6 335 1973 K. Rasmussen.

2. Prediction of right ventricular systolic pressure in pulmonary stenosis from combined vectorcardiographic data. Amer.Heart J 86-318 1973 K. Rasmussen & S Sørland.

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1 Preface

The six papers referred to in this work represent the efforts by me and my co-workers to evaluate and improve the diagnostic methods vectorcardiography (VCG) and electrocardiography (ECG) in various specified conditions involving overload of the right ventricle. In the individual papers the detailed aims and results of each study are presented. This review of current problems in quantification and differentiation of disease by ECG offers a general theoretical background to these 6 studies. Methodological questions which only were touched upon in the individual papers, are discussed in some detail.

The six papers are works along different lines which were thought to be potentially fruitful at the time they were initiated. From a methodological point of view they may be classified as follows:

Group I Paper 1. *Methodological* study.

Group II Papers and 3. *Correlation* studies, searching for simple and combined VCG data which optimally predict hemodynamic data in subjects with pulmonary stenosis and atrial septal defects.

Group III Papers 4 and *Discrimination* studies dealing with the ECG–VCG differentiation of two different disturbances of ventricular depolarization associated with atrial septal defects and pulmonary stenosis from their uncomplicated mother defects.

Group IV Paper 6. *Experimental* study dealing with the ECG changes in an animal model of acute right ventricular overload.

Papers and 3 are mainly concerned with the diagnosis of right ventricular hypertrophy, papers 3 and 6 with the possibilities of detecting right ventricular dilatation in the chronic and acute stage.

In this work the term ECG is used to designate all types of recordings of the electromotive forces of the heart, whereas the term VCG is limited to 3-lead ECG regardless of display form.

During the last decades a revolutionary breakthrough in the surgical therapy of heart disease has taken place. A prerequisite of this development has been the ability of the cardiologist to establish precisely both the type and severity of the lesion present. The tools which the cardiologist uses in this work may be classified as non-invasive or invasive. In general, only the invasive ones have been regarded as reliable and precise enough to satisfy the demands when an operation is being considered. Although they have steadily grown more precise and less hazardous, most of them still involve some risk and discomfort to the patient. They are also commonly time-consuming and expensive and may involve more or less artificial conditions of investigation. It is therefore an important goal in cardiology continuously to reevaluate the non-invasive methods by means of the invasive ones in order to be able to extract as much information from the former as possible. Through this process many non-invasive methods, including ECG, have obtained a "second wind" of interest and applicability.

2 Hemodynamic types of ventricular overload Physiologic-anatomic relationships

An increased demand for work may be imposed on a ventricle acutely or chronically. An acute overload must be coped with by an unchanged muscular mass through an increased myocardial contractility induced partly by an increased sympathetic activity and partly by the Frank Starling mechanism. These two mechanisms are involved both during a primary flow load and during a pressure load.

In the chronic state however the two hemodynamic types of overload differ more fundamentally. First, the lesion may impose an increased resistance to ventricular ejection, thus requiring that the ventricle produces a higher systolic pressure to yield an unchanged stroke volum. In the compensated hemodynamic state this is achieved through compensatory ventricular hypertrophy while both end-diastolic ventricular volume and stroke volume are usually normal[3]. In this stage there is a good correlation between the load and the degree of hypertrophy (paper 2). When hypertrophy no longer can develop ventricular dilatation and heart failure emerge, and the pressure hypertrophy relation is broken.

The second fundamental type of overloading is that induced by volume or flow. This always involves ventricular dilatation. In the compensated state the increased stroke volume is coped with by an increased end-diastolic volume the ejection fraction being unchanged[4,5]. When an upper limit of stroke volume is reached, incompensation occurs, with a decreasing ejection fraction and an increase of the diastolic volume out of proportion to the flow load. Thus, there is a relationship, but not an absolute one between end-diastolic volume and stroke volume (paper 3).

For several reasons, chronic volume loading always involves some degree of accompanying hypertrophy (paper 3)[6]. First, according to the law of LaPlace fiber tension increases during dilatation in proportion to the radius of the chamber. This induces ventricular hypertrophy even when the systolic ventricular pressure is unchanged. Second, the ventricle usually increases the peak systolic pressure in order to eject the increased stroke volume which adds to the hypertrophic stimulus. Third, a normal ventricular outflow tract may be inadequate for the increased flow leading to a further increase in the systolic pressure. The resulting hypertrophy thus reflects both the pressure and the volume increments. Hypertrophy may therefore be regarded as the principal mode of reaction of the heart to both types of chronically increased demand for work. In the heart muscle fibers, hyperplasia does not occur after embryogenesis.

3 Genesis of cardiac hypertrophy

During acute overloading the total and external work of the heart chamber and for each *fiber unit increase. Wall stress, expressed as* force per unit cross-sectional area, and oxygen consumption per weight unit increase concomitantly These two factors are among the possible triggers of the many metabolic and cellular events occurring during early hypertrophy the principal components of these being increased protein synthesis[4 ... 10] This synthesis is generally localized to the specific region under stress. Through a delicate feedback system the process continues to a point where the triggering factors are almost or totally normalized. Thus, there is a fundamental and probably linear relationship between the degree of hypertrophy and the degree of loading.

Although more subtle indices of contractility involving the velocity of contraction, are reduced in hypertrophy[12,13] most studies indicate that the hypertrophic myocardium may create as much force per weight unit as normal heart muscle[8] Several studies indicate that pressure work gives a steeper increase in myocardial oxygen consumption and wall tension than flow work. Accordingly the increase in muscle mass is usually greater in pressu e loading[16]

Since both types of overload probably elicit hypertrophy by the same general mechanisms, one would think that the process would have a similar distribution in both conditions. Some observations indicate, howeve that pure pressure work gives a symmetrically distributed hypertrophy and a flow load a mo e assymmetrical one *Such differences may be elevant to the* interpretation of ECG patterns seen in volume loading (paper 3)

Several factors may disturb the dynamic state of equilibrium between chamber stress and the anatomical esponse to it.

a) Both the development and the regression of hypertrophy require time In human at least months of stable loading are probably needed.
b) The anatomic-physiologic relationship seems generally to be closer in congenital than in acquired heart disease Possible explanations may be a greater potential for compensatory hypertrophy in the former and a greater tendency to concomitant fibrosis in the latte
c) Several associated diseases of the heart may modify the hypertrophic response This applies especially to coronary heart disease and hypertrophic cardiomyopathy The latter is characterized by a muscle mass out of proportion to the load. *The best established example* is hypertrophic obstructive cardiomyopathy another may be the hypertrophy in subjects with Turne phenotype and pulmonary stenosis described in paper 4

In addition, methodological problems of a more general nature arise in practical comparisons of anatomy and physiology

4 Anatomic-electrocardiographic relationships in hypertrophy and dilatation

Several changes in the electrophysiology of the heart may occur when the muscle mass of a heart chamber increases.

I Myocardial factors.

a) *Altered membrane potential*

Several factors may change the distribution of electrolytes across the heart cell membrane thus altering the membrane potential and action potential. This has commonly been regarded as unimportant during stable hypertrophy but systematic changes in myocardial potassium content have been described in this state[19]

b) *Increased dipole magnitude*

This is the principal factor responsible for ECG changes during hypertrophy The instantaneous dipoles generated during depolarization are primarily dependent of the area of the bordering cell membrane surface between depolarized and non-depolarized myocardium. Therefore, the dipole magnitudes are proprotional to cell surface circumference and cell radius. If fiber mass of cylindrical cells is increased solely by an increase in cell radius, the dipole ought to be proportional to the square root of the weight increment. However most empirical studies indicate a linear regulationship between muscle mass and dipole magnitude and this linear relationship has been most frequently used in diagnostics[20]

This increase in dipolar activity in the hypertrophic region disturbs the equilibrium between the various parts of the heart in the formation of the resultant ECG recorded at the body surface The QRS resultant is dislocated in the direction of depolarization of the hypertrophic region, whereas the ST T segment is dislocated in the opposite direction.

c) *Altered direction of depolarization*

Hypertrophy is often accompanied by true conduction disturbances. But even when the conduction system is intact, the sequence of depolarization may be gradually and slightly changed during developing hypertrophy This is mainly a consequence of a delayed depolarization in the hypertrophic region due to the longer conduction distance. The increased depolarization distance is probably not a consequence of fiber elongation since sarcomere length does not exceed a critical length of about 2.2 μm Thus, sliding and rearranging of muscle fibers, in addition to the increase in diameter are probably the most important factors. The consequence is a more tangential spread of depolarization through the hypertrophic myocardium than the normal more direct endocardial to epicardial spread.

To my knowledge no quantitative empirical data concerning this process are available The hypothesis, based on the analogy with nerves, that thick fibers conduct faster than thin, has not yet been given empirical support[22 23]

Especially during chronic flow loads the QRS duration may exceed the upper normal limit because of the dilatation alone without any demonstrable branch block (paper 3)[2 25]

d) *Brody-effect.*

The ventricular end-diastolic volume, i.e. the volume present during ventricular depolarization is increased in several types of overload. The 'Brody-effect refers to the physical consequences of increasing the amount of a good conductor (blood) within a shell formed electrical generator (heart) Such a dilatation is expected to potentiate the normally oriented forces and suppress the tangential ones[26] The effect has been confirmed in model experiments[27 28] but empirical data as to its importance during dila-

tation of the human heart have been sparse. Paper 6 attempts to supply a few such data from dogs, and paper 3 offers a less direct approach to the problem.

II. Other more indirect mechanisms of ECG changes during hypertrophy and dilatation.

a) *Reduced electrode to epicardial distance*

This factor may alter the ECG in an unpredictable way, *varying with constitutional variables* and lead system. Orthogonal VCG systems aim to eliminate this factor.

b) *Myocardial hypoxia.*

Hypoxia, induced by the prolonged diffusion route for oxygen, has often been suggested as a factor in the genesis of the ST-T changes during hypertrophy. Hypoxia may certainly occur in acute overloading and in several cases *of chronic* overloading, and may then also contribute to the ST-T changes, but it is probably not a major factor in uncomplicated hypertrophy[4,29,30] where *the ST-T changes are essentially secondary.*

Many reports and terminologies have been been based on the concept that there is a direct association between the physiologic stress and ECG unrelated to anatomic changes[18]. *In my* opinion this concept has a poor empirical support. In chronic overload the influence of the load on ECG is primarily mediated through *anatomical changes.*

In summary, the effect of hypertrophy and dilatation on the ECG may be caused by several mechanisms. For all of them, the exact quantitative *nature of the anatomy-ECG relationship is* unknown, and the individual variability of the response is probably large. The increased dipole magnitude should be regarded as the principal mechanism, being modified to some extent by the others. These elements of uncertainty make it impossible to predict quantitatively the net effect of all factors. This leaves both degree, type and constancy of the ECG changes during ventricular hypertrophy open to empirical and primarily clinical studies. A wealth of such empirical studies dealing with the different ECG patterns in various types of heart disease is available. They have mostly been confined to a search for ECG variables which linearly reflect the degree of anatomic or physiologic changes.

The classical method of correlating ECG data with anatomy by means of autopsy materials involves several difficulties[32]. First, autopsy materials are commonly highly selected as to the *severity of disease. Second, the patients who die* may have a higher incidence of complicating factors than the average reference population. Third, the period immediately preceeding death *may involve fluctuations in many factors which* influence both anatomy and ECG. A fourth obvious problem is the scarcity of autopsy data in several types of heart disease. Fifth, autopsy *methods may differ and may be difficult to inter*pret. It is difficult to evaluate only the muscular components of the heart. Various degree of fibrosis may reduce the observed correlations. *These factors may explain why studies of ECG-*anatomic relationships have yielded rather modest results and why many workers, including the author, have turned to the direct comparison of *physiology and ECG. In spite of the many* methodological problems in transferring physiology to anatomy, this approach has in many studies showed better correlations than in post-*mortem studies. In addition, a load quantification* is commonly the most relevant information needed for clinical decisions. The over-all relationship between physiology, anatomy and *ECG is summarized in Fig. 1.*

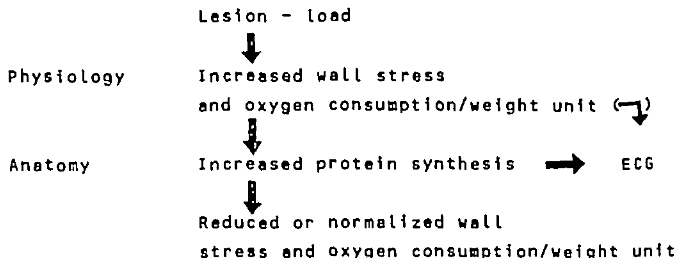


Fig. 1. Overall relationship between physiology, anatomy and ECG in ventricular hypertrophy

5 Conduction aberrations and the ECG diagnosis of ventricular hypertrophy

Interpretation of ECG in terms of anatomy is fundamentally dependent on a normal depolarization sequence. It is well established in clinical practice that conduction defects in the bundle branches or accessory atrioventricular conduction may disturb or totally invalidate the ECG diagnosis of hypertrophy. Another example is the depolarization aberration seen in endocardial cushion defects (paper 5). In this condition, however, the distortion does not profoundly disturb the evaluation of hypertrophy. A last example may be the "Turner phenotype ECG" described in paper 4, where the diagnosis of hypertrophy is profoundly altered by the changed depolarization sequence. Thus, conduction disturbances are of interest because they necessitate the revalidation of ECG criteria of hypertrophy. They are, however, also of interest per se. This is especially so if the disturbance is linked to other clinical data of interest, e.g. to prognosis. Both these elements are present for the two abnormalities of depolarization discussed in the present work (papers 4 and 5).

It is often difficult to establish reliable diagnostic criteria for a conduction disturbance. In order to prove its presence one must either make histologic and electrophysiologic analysis, which is difficult in living subjects, or one must utilize a firm link between the disturbance and some unequivocal anatomic or clinical conditions. This method was the only one available in the present studies (papers 4 and 5). In fact, their importance lies not in the improved diagnosis of the conduction disturbance but in that of the associated defects (ASD$_I$ and Turner phenotype). While the development of hypertrophy is a continuous process, the conduction abnormality is an all-or-none phenomenon. Therefore, correlation studies are appropriate in the analysis of the former (papers 2 and 3) and discrimination studies in the latter (papers 4 and 5).

6 Methods for improving both the quality of the ECG tracing and its relevance to the diagnosis of hypertrophy and dilatation

During the la t decades several studies in this field using conventional 12 lead ECG have appeared. Both in these and in those applying other lead ystems, isolated criteria, commonly selected at random, have often been compared with anatomic and physiologic data. In this situation three lines of possible improvement seemed natural.

a) Testing of a wide range of criterie including all the main components of the ECG signal In this manner prejudiced decisions regarding the relative importance of different data based on some a priori hypothesis could be avoided.

b) The combination of data, in order to pick up the maximal cumulative information present in the total ECG signal. These methods are dealt with in Section 10c.

c) Improvement of the ECG recording in a manner which more accurately reflected the phenomena taking place in the myocardium. The following such approaches have been discussed as potentially fruitful

1 Substitution of 12 lead ECG with an orthogonal 3 lead system The main principles behind such systems are discussed in Section 7

2 The multiple dipole model involving the use of multiple surface leads and a computer programmed to sol e the equations connecting the surface recording to the dipole generators of the heart[2] The method seems promising but was not available for this work

3 Several other complex physical model which are more accurate physically have been described. Among these are moving dipole and multipole models Multipoles are said to account for between 10 and 40 per cent of the electrical activity retrieved at the body surface[35] Up to now no one has demonstrated how this information may be transformed into diagnostic use Future advance may however lie along this line.

For the present work, the simultaneous application of methods a, b and c 1 was chosen.

7 The orthogonal 3-lead ECG

a. *Criticism of the 12 lead system.*

The conventional 12 lead ECG system has been developed through a casual historical process, and not as a result of an accurate biophysical analysis. The most important deficiencies of the system may be summarized as follows.

(1) Much of the information obtained is highly redundant, particularly that applying to the extremity leads[38] but the chest leads also may be constructed from each other with great accuracy[4]

( ) The leads, in particular the precordial leads, are in a complicated and largely unknown manner dependent both on the heart as a whole (th single equivalent dipole) and on the part of the heart closest to the lectrode (proximity effect) It is thus still obscure how information concerning specific parts of the myocardium may be systematically extracted from the 12 lead ECG

(3) In spite of the superfluous inform tion recorded by th system, the lead fields of many leads are such that some parts f the heart may not be adequately represented in any lead[38] This applies especially to the posterior parts of the heart

Another consequence of the inhomogeneous lead fields is that electrical phenomen in the heart which diffe greatly both in magnitude and direction may be recorded similarly in a lead[38] Thus, both the strength and the direction of a dipole may be difficult to assess.

(4) The individual 12 leads are not balanced. A dipole of equal strength in the heart directed along the field lines of different leads may give deflections greatly differing in magnitude.

These deficiencies of the 12 lead system are all consequences of well-established facts. the heart is no point generator the body is an inhomogeneous conductor with an irregular surface and some parts of the heart are close to the body surface. I spite of these problems the 1 lead ECG has bee and will continue to be useful practical method because of its abundant empirical foundation. However the deficiencies of the system are particularly important when the ECG is used for quantitative evaluation f myocardial mass and volume The inhomogeneous lead fields may cause an addition l myocardial mas to have quite diff rent possiblities of bei g detected, depending on its localization

b) *Principles of the 3 lead system*

The single equivalent dipole hypothesis, which is the simplest and most widely used model in ECG interpretation, states that with regard t surface leads, the heart behaves as if its electrical activity is confined to one simple equivalent dipole located in the electrical cente of the heart. All changes in the ECG could then be described as variations in strength and direction of this dipole If this hypothesis were correct, ll information present in surface ECG would be available in three leads directed along the three body axes.

Fo reasons alluded to above, this hypothesis is only partially correct[35 39] The inaccuracies are however to some extent corrected fo by the body itself Since the heart is filled with a good conductor surrounded by poor ones, the body surface is in the electrical sense removed from the heart, rendering the dipole hypothesis more tenable than is often held[38]

The special design of the orthogonal 3 lead systems aims towards further adjustment f the inaccuracies of the dipole hypothesis. Leads are sought which have homogeneous field througout the heart[38] or in other words, have a niform lead vector in ll parts of the heart[40] This is achieved by careful selection of electrode positions and by the combination of several dif ferent electodes into one lead The main effect

of the design is to secure a one to-one relationsh p between dipole magnitude and direction in the heart and the corresponding d pole recorded by the system An increased muscle ma in some part of the heart, g ing a certain change in the equivalent dipole may then be more accurately reflected Similar advantages may be present in the recording of dilatation and conduction disturbances. Furthermore when the disturbance from extracardiac factors is reduced, one may expect a better separatk n between normal and abnormal tracings [2]

The orthogonal 3-lead systems are furthermore designed to satisfy the following requirement

(1) The three leads should be electrically perpendicular t each other in the heart, a dipole directed along one of the lead a es in the heart thu being represented only in this lead.

( ) The leads hould be mutually balanced so that a un t dipole in the heart gives the same deflection in all leads when directed alo g the lead a is

When these requirements are satisfied the synthesis of spatial dipole magnitude and direction becomes a matter of applying the Pythagorean thorem.

The concentration on the recording of dipolar electrical activity by the 3-lead y tem may be thought to result in a los in the ability to record poles of higher order However also the 3-leads ystem probably have some ability criterion of a lead y tem lies in it practical diagnostic performance and not in the physical properties of the ystem. Deviations from the physical ideal may in some sit ations actually tend to improve a lead ystem[44]

8 Choice of 3-lead system

It is generally accepted that the choice of a VCG system involves a selection of the optimal balance between accu acy and practicabil ty An accurate lead system with electrodes has been designed by Brody and Arzbaeck r[4] Ba r et al[44] have established that a minimum of 4 properly placed electrodes requir d to deter mine consistently the total body QRS surface potential distribution For common clinical prac tice that many electrodes would be too time consuming On the orther extreme the simple and early systems (cube equilateral tetrahed on) have largely been abandoned because of ther lack of orthogonality and balance and poor lead field properties. Most workers today agree in that the optimal balance between these extremes lies n the use of 7 to 10 lectrodes The Frank system[4] which is th most idely used has electrodes while the axial system designed by M Fee and Pa ungao[4] nd used in this work has 9 electrodes Othe good tems a th SVEC III and the Helm sy tems All these sat sf fairly well the abov -mentioned requ rements Sev r l studies are a ailable which compare these sy tems with regard to thei biophysical properties [40] The esult are conside bl with the criteria selected fo evaluation and with the priority giv n to d fferent criteria In gene al the axial sy tem has yielded sl ghtly bette results than the F nk system Stud es are also available which compare ecordings made w th d fferent systems These slight differences in the f.n of the axial system ma in part eflect the d f f ent sampling of lectrical forces on the thorax

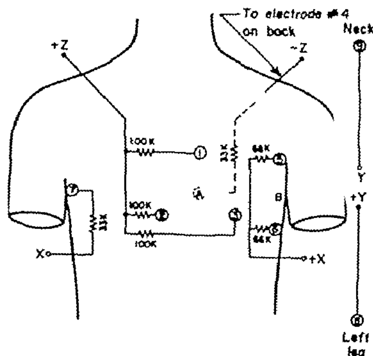


Fig The ial lead stem

Other differences between the two systems are discusses in paper 1

Partly because of these considerations and partly for historical reasons the axial lead system was chosen for the present work. The lead system is presented in Fig 2.

Although orthogonal lead systems had been used for several years at the start of the present work, no study on their reproducibility was then available Such a study was therefore conducted (paper 1)

It ha been maintained that the strength of lead Z in the a ial system is too high and that it should be reduced by about one third[13 - 14] Since the theory behind this correction procedure did not seem to be generally accepted and since it would necessitat a resampling of all materials, the procedure was not adopted in the present work This choice had only potential influence on the diagnostic performance of angular and spatial data.

The ultimate evaluation of a lead system should be made from its ability to diagnose the most important clinical conditions. To my knowledge truly relevant studies of this main question re still lacking An impressive comparison has been made by Gamboa nd Hugenholtz testing 12 lead ECG the cube, and the Frank ystem on right entricular hypertrophy Twelve lead ECG and various VCG ystems have been compared in several studies a to their ability to diagnose myocardial infarction[44] Different orthogonal VCG systems have however to my knowledge not been compared. The main re quirement for such comparisons is that maximal information of one system should be compared with the corresponding maximum of the other The discussion about the optimal lead ystem will continue until such studies are presented.

9 VCG recordings and variables

The most important technical equirements of an ECG ecording system are as follows

1 Adequate skin preparation, in order to reduce skin-electrode resistance
2 Adequate electrodes.
3 High impedance of the input amplifiers in order to reduce the influence of skin-electrode resistance[49]
4 Adequate frequency response of the recorde with neutral damping.

These equirements were reasonably fulfilled in the present study

Traditionally 3-lead ECG was displayed as planar loops on an oscilloscope and photographed with Polaroid film. This form gives good presentation of loop rotation and form and contains information about ectorial spatial magnitude and angles not readily available from the scalar leads. The drawback are the less exact time marking and th discontinuous amplitude recording (paper 1) During the last years high speed simultaneous recordings of scalar leads have been preferred by most workers[49] In the present work both types of ecordings w re used

In order to characterize an instantaneous vector completely three data are needed, either in the form of Cartesian (X, Y and Z) o spherical (magnitude azimuth and elevation) coordinates There has been an increasing tendency n vectorcardiography to choose spherical data especially has the maximal spatial magnitude been a popular index of ventricular hypertrophy This popularity originated in its seemingl logical nature and in the many studies which indicated its superiority [49] This concept is challenged in the present work (papers and 3). The spherical coo dinates have perhaps been chosen at the expense of the mo e easil retrievable Cartesian coordinates on too loose evidence In paper the two types of data were compared and in the other studies the Cartesian data wer preferred The maximal ector spatial magnitude was used as one among many potentiall useful ariables

The manner in which QRS data ar sampled is also subject to great differences Some workers divide the segment into parts of equal length Implicit in this choise is the belief that all part of the depolarization sequence change proportionally during variations in the QRS duration An alternativ method is to sample with constant time intervals assuming that the most common cause of ariations in QRS duration lies in the terminal part. Both of these views ar probably partly correct In this ork constant time intervals were chosen Simila problems exist with rega d to ST-segment sampling in this ork a single ST-segment point 0 10 sec. from the QRS end was chosen

10 Evaluation of diagnostic criteria

The first question which ordinarily arises in the diagnostic process is the differentiation between normality and abnormality[42]. In the present six studies this problem is only partially touched in the two correlation studies, since the normal state constitutes the lower extreme of "degree of disease". The emphasis of the studies lies mainly in a later clinical situation, when a subject has been identified as abnormal and the diagnostic possibilities have been restricted to one or two.

Diagnostic tests may either function as discrimination tests, with the aim of indicating which of a given set of diagnoses is most probable, or as correlations tests, aiming at quantification of disease. The two types are generally used at different steps in the diagnostic process: the type of disease must be known before an exact quantification is of interest.

a) *Discrimination among groups (papers 4 and 5)*

The simplest test aims to allocate subjects to one or another group of diseases, or above or below a certain level of disease. The most common variant of the test is the differentiation between 'normal' and 'abnormal'. The problem is to find data and criteria which separate the groups as completely as possible. The results are evaluated from the fraction of false negative and false positive classifications, commonly in terms of specificity and sensitivity. The two indices are profoundly linked together and are sometimes combined in a performance index, as applied in paper 5. If the practical consequences of misclassification one way differ from the other, these may be built into the rule, thus introducing a subjective element in the classification. The question of correct use of a priori probabilities also arises[44].

These problems were not present in paper 4 since several criteria were found which separated the two groups completely. The paper illustrates another use of discrimination studies, namely its contribution on the definition of diagnostic entities and to the clarification of biological differences.

b) *Quantification of pathology (papers 2 and 3)*

In several clinical situations, however, this simple two-group classification may not be sufficient and may in fact serve as a restriction to knowledge. Instead of using fixed cut-off levels, one may take diagnostic advantage of the entire distribution of continuous variables. In this manner more information may be extracted from the method. Commonly this is done by searching for optimal correlations between ECG data and an independent key method which most adequately expresses the "degree of disease". This method is applied in papers 2 and 3.

The most important problem with this method is what requirements should be made regarding quality and quantity of data. These two entities are of course competitive. In the present work comparatively small but well-defined samples were used.

Another problem is how great the difference in performance must be in order to be accepted as an improvement when two variables from the same material are compared. In such comparisons the sample dependence of all correlations has often been overlooked[*]. This problem can be dealt with using standard statistical techniques.

Comparisons of diagnostic criteria from different materials are dangerous. Direct comparison of correlation coefficients and standard errors of estimates may be misleading since these variables are highly dependent of the range

of observations. The best method is probably a simultaneous consideration of both these indices.

Linear regression analysis, the most common method in correlation studies was also used in the present work (papers 2 and 3). It is however possible that non-linear correlations might have improved the results.

c) *Data combinations.*

One further step towards the extraction of maximal diagnostic information is the use of data combinations. The principle has a long tradition in electrocardiography. The most common example is two-data combinations. Whereas the one-datum diagnostic process may be illustrated by observations along a simple line observations in two-data diagnostics may be represented by points on a plane with one variable oriented along each axis[44]. A common example from ECG is the diagnosis of left ventricular hypertrophy from the sum RV5+SV1. When three data are used, each case may be represented by a point in a three-dimensional space. Further expansions of the principle are difficult to visualize but have mathematical validity. Each case is then represented by a point in an n-dimensional space n being the number of variables applied.

Traditionally this method has been applied with an intuitive selection both of variables and of weight factors assigned to each variable (point score systems). The logical method, in a field with many useful and highly intercorrelated variables, is, however to select both variables and weight factors on an entirely empirical basis. This selection is handled by multivariate statistical methods. In papers 3 and 5 step-up procedures were used which first select the single variable yielding most information and then stepwise add other variables which contribute substantially to the sum of information. Each variable is given a weight in proportion to its relative contribution. These statistical methods are only practicable when a computer can be used to solve all the matrix equations involved.

The main problem with this method is that a large amount of data is equired since such calculations based on small samples tend to be misleading. The problem has been discussed extensively by Pipberger and his group[43, 44]. They have given somewhat differing advice concerning the maximal number of variables which can be used with a given amount of cases. First they allowed as many variables as the square root of the number of cases later[4] the maximum level was changed to 1/20 of the number. Applying these standards the figures of the three present papers using these methods were

	No cases	Square root	1/20	No variables used
Paper 1	35	6		5
Paper	50	7	5	4
Paper 3	103	10	5	and 4

Thus, the later requirement is not completely fulfilled in all the present studies. These requirements, however are partly of an empirical nature and are particularly derived with regard to discriminant analysis. The point is, nevertheless essential. All diagnostic criteria derived from samples are sample-dependent, and criteria derived by *multivariate methods are especially* so. All criteria should therefor preferably be tested on new data samples. This is carried out only in paper in this work. Conclusions regarding the results must therefore be drawn with special care

11 Aims of the present studies

In the above presentation several problems are left open. The main aim of the present work was to test the ability of VCG to contribute to the clinical diagnosis of diseases involving the right ventricle. The emphasis was placed on the selection of optimal VCG criteria. The method was regarded as supplementary to rather than opposed to conventional ECG. Therefore only papers 2, 4 and 5 contain comparisons of these two methods. The work was concentrated on problems where the documentation in current literature seemed incomplete and where the available clinical material seemed adequate.

Group I (paper 1)

As discussed, knowledge of the reproducibility of orthogonal ECG is essential for its use in exact diagnosis. At the time this work started, no such study was available. Therefore, in a small series of 70 subjects, the reproducibility of the axial lead system used in this work was tested. Before the publication of the results two similar studies of the Frank system appeared, rendering a comparison of the two lead systems possible.

Group II (papers 2 and 3)

Clinical conditions in which the abnormal load on the heart was confined to the right ventricle and in which the hemodynamic load and thus probably its hypertrophic response could be precisely quantified were selected. In pulmonary stenosis the right ventricle is subjected to an almost pure pressure load without associated volume loading, and in atrial septal defects the volume load dominates over a small to moderate pressure load. For these two defects correlations between hemodynamic data and simple and combined VCG data were sought.

Group III (papers 4 and 5)

As discussed, abnormal sequences of depolarization are of interest partly because of their invalidation of common ECG diagnosis of hypertrophy and partly because the abnormal depolarization may be linked with prognostic and anatomic traits. One such abnormality was studied for each of the "mother defects"; the problem raised was that of discrimination between the uncomplicated defect and a defect with aberration of depolarization. One study (paper 5) dealt with the classical problem of differentiation between atrial septal defects of primum and secundum types. Although several authors have described the different VCG patterns in these conditions, no systematic study of optimalization of VCG criteria was found. Such a study was therefore designed. The second study (paper 4) describes an original discovery made by Dr Sørland and me. We recognized that it was possible to define a subgroup of patients with pulmonary stenosis, both clinically and electrocardiographically. Soon afterwards similar observations were reported by others.

Group IV (paper 6)

The role of right ventricular dilatation in the genesis of ECG changes both in acute and chronic disease has been much debated. At the start of the present work no study was available which treated this problem in an adequate experimental manner. A study in dogs was therefore set up in order to elucidate this theoretical question. The problem has, however, a close clinical counterpart of major practical importance, namely pulmonary embolism. The study is therefore relevant to the mechanism of ECG changes and selection of ECG criteria in this condition.

12 Synopsis of the results

The results of the individual studies are summarized in the individual papers. This synopsis gives some comments with more relevance to the work as a whole and to possible applications of the results.

Paper 1

This study establishes the reproducibility of measurements adopted in the other studies. It emerged that some types of measurements, i.e. amplitude optima and directions of loop rotations, tended to be highly reproducible whereas instantaneous vector data tended to be much less so. This correlated well with the fact that the former type of data was found to contain most diagnostic information in studies and 3

Paper 2

This study embodied the main ideas of the work. VCG data were found to have some superiority over conventional ECG data in the diagnosis of right ventricular hypertrophy as evaluated by the ability to correlate with right ventricular systolic pressure. A substantial improvement of diagnostic performance was obtained when VCG data were combined by a multiple regression analysis. The results were in principle found to be reproducible in a second patient sample. The accuracy of the pressure prediction was so good that a correct decision regarding operation would have been made in almost all instances by means of this rapid and noninvasive diagnostic test

Paper 3

This study followed the same lines as study 2. More emphasis was put on the simple VCG data, since previous reports differed greatly in opinion as to the value of these and conventional ECG was not applied. In general, a good pressure prediction but a poor flow prediction was found. The multiple regression analysis was applied with greater care than in study but the results nevertheless indicated an improvement. The method seemed able to exclude right ventricular hypertension with great precision in patients with atrial septal defects, thus possibly reducing the demand for preoperative heart catheterization. As a by product a comparative analysis of the patients in studies and 3 was made. This indicated that the primary effect of ventricular dilatation is to sensitize the ECG to the effect of pressure. This effect may possible be mediated through the Brody effect discussed above

Paper 4

This paper describes the ECG and VCG of patients with pulmonary stenosis exhibiting the Turner phenotype with normal chromosomes syndrome also termed Noonan's syndrome. The study establishes, for the first time in a precise manner the character of the depolarization abnormality in this subgroup. This specific ECG-VCG pattern may help to diagnose additional defects in such patients and may also help the surgeon to select an open technique for operation, since the valve stenosis may be more resistant to closed methods in these patients

Paper 5

In the search for VCG data which differentiated atrial septal defects of the primum and secundum types, the conventional ECG axis was used as reference method. Since this was rather accurate no great improvement could be expected. Nevertheless several criteria were found to give a significantly better prediction of the type of defect. The study also led to a more exact definition of the depolarization abnormality found in endocardial cushion defects. The results

of data combination were modest. The main result of the study is that a VCG analysis in some patients allows a more exact preoperative diagnosis to be made. This gives the decision to operate a firmer basis and better prepares the surgeon for the problems facing him during operation.

Paper 6

The induction of acute right ventricular hypertension and dilatation in dogs led to a well defined pattern of ECG changes. The changes occurred only at an advanced level of pulmonary artery obstruction and then rapidly reflected the changes in the degree of obstruction. Both these features correlate well with clinical observations in man during acute pulmonary embolism. The results may help in the analysis of observed ECG changes in this condition and may be of value in the search for ECG–VCG criteria which may reflect the disease. The study also has some theoretical implications as to the effect of ventricular dilatation without concomitant hypertrophy.

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Morphological and Biochemical of Orally Administered Rapeseed Oil on Rat Myocardium

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Preface

Oil from the seed of different Brassica varieties — rapeseed oil — has been produced in Sweden in increasing amounts since world war II. The crop yield is at present just below 300 000 tons per year. Rapeseed oil was used for human consumption in Sweden in small and varying amounts in the 1940s. After the middle of the 1950s however it was used regularly in large quantities, above all as an important part (about 30 %) of the fats in margarine.

Since the 1940s reports in the literature have revealed that feeding of large amounts of rapeseed oil to laboratory animals and swine causes some growth retardation and changes in the testes, adrenals and liver. These effects have been considered to be related to the high content of erucic acid. This is a long-chain fatty acid with 22 carbon atoms and one double bond, and is therefore referred to as a C22.1 acid. The above observations did not bring about any actions as far as consumption in humans was concerned until the second half of 1970. By this time there was evidence not only that feeding of rapeseed oil to rats and several other animals was consistently followed by lipoidosis and fibrosis of the myocardium, but also that it interfered with mitochondrial function, with reduced oxidation and ATP synthesis, in the rat.

Since such a basal function as the energy production by the myocardial cells was involved, the Swedish margarine industry after consultations with its experts, decided in August 1970 to reduce the content of rapeseed oil in margarine from 30 % to a maximum of 15 % of the fat. Since other oils containing C22.1 acids could also be expected to have the same effects as erucic acid, it was decided in 1971 to limit the content of C22.1 acids in margarine to a maximum of 7.5 % of the total fatty acids. In the latter part of 1972, when new varieties of rapeseed were harvested, the oil recovered had a lower erucic acid content, below 20 % and thus a further reduction of the C22.1 content in margarine to a maximum of 5 % of the fatty acids was established. Analyses on margarine in Sweden performed during 1973 at the National Food Administration did not show any levels of C22.1 acids above 5 %.

In 1970 the Swedish National Board of Health and Welfare at the request of the Swedish margarine industry appointed a committee of experts to examine any evidence of possible toxic effects of erucic acid in man, and to investigate whether the findings in the animal feeding experiments could be applied to human consumption of erucic acid. In a preliminary report in 1971 this Expert Committee concluded that the actions taken by the margarine industry were sufficient, as there was no direct evidence of untoward effects of consumption of erucic acid in man.

After reviewing the scientific data, the Swedish Medical Research Council decided in 1971 that there was a need of confirmation of some of the results and also of an extension of the animal experiments to include effects of low level consumption of erucic acid more equivalent to the amounts used by humans. The Medical Research Council initiated a series of projects concerning the morphological, biochemical metabolic and physiological effects of erucic acid feeding in rats. These studies have been directed by a working party organized by the Medical Research Council and the National Food Administration, which have also provided the financial support. The results are included in this volume as separate reports by the various investigators. The conclusions arrived at by the whole group are presented in the general summary

Stockholm, March 1975

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Physiopathological Effects of Rapeseed Oil A Review

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Abstract

Rapeseed oil has a growth retarding effect in animals. Some investigators claim that the high content of erucic acid in rapeseed oil alone causes this effect, while others consider the low ratio saturated/monounsaturated fatty acids in rapeseed oil to be a contributory factor

Normally erucic acid is not found or occurs in traces in body fat, but when the diet contains rapeseed oil erucic acid is found in depot fat, organ fat and milk fat. Erucic acid is metabolized in vivo to oleic acid. The effects of rapeseed oil on reproduction and adrenals, testes, ovaries, liver spleen kidneys, blood, heart and skeletal muscles have been investigated Fatty infiltration in the heart muscle cells has been observed in the species investigated. In long-term experiments in rats erucic acid produces fibrosis of the myocardium. Erucic acid lowers the respiratory capacity of the heart mitochondria. The reduction of re spiratory capacity is roughly proportional to the content of erucic acid in the diet, and diminishes on continued administration of erucic acid.

The lifespan of rats is the same on corn oil, soybean oil, coconut oil, whale oil and rapeseed oil diet.

Rats fed a diet with erucic acid or other docosenoic acids showed a lowered tolerance to cold stress (+4 C).

In Sweden erucic acid constituted 3—4 % of the average intake of calories up to 1970 compared with about 0.4 % at present.

Introduction

Rapeseed oil is produced and consumed in many countries. At present, the world production of seed from rape and its relatives amounts to about seven million tons per year and rapeseed ranks fifth in the production of vegetable oil, preceded by soybeans, cottonseed, peanuts and sunflower seed. The oil differs from most edible fats and oils as having low level of saturated fatty acids and a high level of erucic acid (about 30 per cent in Canadian rapeseed oil and about 50 per cent in European rapeseed oil). Erucic acid consists of 22 carbon atoms with one double bond (Δ 13). Rapeseed oil also contains 5—10 % eicosenoic acid (C 20:1). Through plant breeding of rape seed the content of erucic and eicosenoic acid has been lowered to close to zero in the oil (canbra oil) The nutriti e value of rape seed oil has been examined for a long time These studies were intensified 1970, when t was proved that rapeseed oil induces changes in the myocardium.

Effect on growth

Rapeseed oil has a growth retarding effect on rats (73 15 67 17 13 57 58, 7), swines (62, 46), ducklings (78), guinea-pigs (34 78), mice (34, 78), hamsters (78) and turkeys (56, 69 70). Growth was retarded in chicks receiving a diet with 25 % rape seed oil (34), but not when the diet contained 4 or 8 % rapeseed oil (71). In a third experiment growth decreased with increasing amounts of rapeseed oil in the diet (5 10 and 15 %) (80). In some experiments with

rabbits rapeseed oil retarded growth (5) but not in others (34 88). No retardation of *growth has been observed in dogs receiving* a diet with rapeseed oil (34 46)

Rats eat less of a diet containing 20 % rapeseed oil compared with one containing for instance 20 % peanut oil. The depressed appetite is not caused by an organoleptic factor (77 78), but involves an effect on hypothalamus (11) The energy content of rapeseed oil is probably the same as that for other oils *(73 78)* The unfavourable growth obtained with rapeseed oil diets is caused by the erucic acid content of this oil (76, 38, 13). The unbalance in the ratio saturated/monounsaturated fatty acids may also be of importance for growth (53 12, 66, 79).

No growth retarding effects have been observed in experiments with rats receiving canbra oil in the diet (64 44 4 66) and neither was growth affected by adding saturated fat to a diet containing canbra oil (44).

Absorption and digestion of rapeseed oil, canbra oil and some fatty acids

In rats rapeseed oil is absorbed more slowly than other fats and oils, which normally occur in our diet (47 75). The digestibility of rapeseed oil has been determined in several experiments on rats. Values between 58 and 95 ha e been obtained (17 34 16, 65 54). Wistar rats digest rapeseed oil much better than Sprague Dawley rats (16). The low digestibility of rapeseed oil is due mainly to its high content of erucic acid (35 38). The digestibility of canbra oil is comparable with that of peanut oil, 95 and 92 % respectively (65).

The digestibility of rapeseed oil has also been examined in studies on chicks (42, 72, 80), rabbits (34 88), lambs (87), swine (61 46), puppies (46) and guinea-pigs (46 34) Homo digest rapeseed oil to 99 % (52, 48).

Bergström et al (26) ha e proved that in human thoracic duct lymph lipoproteins the absorbed erucic acid is transported unchanged *in chylomicrons and very low* density lipoproteins (VLDL) The incorporation of erucic acid in different lipid classes of chylomicrons and VLDL is different from that of oleic and palmitic acid (higher content in cholesterol esters and less in phospholipids).

Metabolism of erucic acid

Erucic acid is not naturally found in animal tissue lipids. Rats fed rapeseed oil diets show less erucic acid and more oleic acid in their tissue lipids than in the diet. Metabolic *conversion of long chain fatty acids* from dietary rapeseed oil to oleic acid has been suggested for a time (36 45 43) and is now experimentally proved (30, 31 60).

By use of labelled (radio-active) fatty acids, it has been demonstrated that erucic acid is oxidized at the same rate as oleic acid but the yield of this oxidation is lower (9).

Changes induced by rapeseed oil and canbra oil

Effect on body fat and milk fat

The dietary fat affects the composition of body fat. The content of erucic acid in organ and depot fat after rapeseed oil feeding has been examined in rats (81 82, 45 86, 57 58) and swine (83) In rats ovary and adrenal fat (84 86) and in swine plasma and adipose tissue fat (83) are most influenced by dietary erucic acid. Erucic acid is mainly incorporated in triglycerides and in lesser amount in phospholipids (27 10, 45 63 28) with the exception of adrenals and ovaries in which it is incorporated mainly in the cholesterol ester fraction (37 33 36, 84).

Milk from rats, fed a diet containing rapeseed oil, contains eicosenoic acid and erucic acid (14 10).

Effect on adrenals

The adrenals in rats fed rapeseed oil diet were larger and contained more fat than the adrenals *from control groups* (32, 33 37 2, 4 5) The adrenocorticotrophin-induced synthesis of prostaglandins in vitro was *substantially lowered in adrenal homogenate* of rats fed a rapeseed oil containing diet compared with rats fed a corn oil containing diet (79)

During cold stress (+4 C) the cholesteryl erucate was badly utilized for the synthesis of steroid hormones and the plasma corticosterone level was lower in animals fed ethyl erucate than in the ones fed olive oil (85). It has been shown, that dietary intake of docosenoic acids (C 22:1), e.g. diets containing rapeseed oil or partially hydrogenated herring oil, in the rat was associated with low survival rates in the cold (+4 C) (18, 20).

Effect on reproduction

In reproduction studies on rats it was proved, that erucic acid and oleic acid in the diet may cause a reduced fertility Most of the rats became pregnant after mating and the young ones were born alive, but the mortality of the offspring was high because of deficient mammary development and lactation in the mother (39). In a reproduction study involving three litters of one generation rats, no difference in the number of animals successfully weaned was recorded, although the young rats fed rapeseed oil were of less weanling weight than the ones of rats fed corn oil (13) In a reproduction study over four generations the rats fed rapeseed oil continued to reproduce, but they had fewer and smaller offspring than rats fed other oils (14).

Effect on liver

Two months on rapeseed oil diet did not cause any histological lesions in the liver of rats (66), *but after 1—2 years an indication* of fatty infiltration and degeneration in the central parts of the lobes were noticed (74 78, 4 5).

Cirrhotic changes in the liver of ducklings could be seen after three weeks on a diet containing 30 cal% rapeseed oil (3). In experiments on ducklings and guinea-pigs the effect of isocaloric oil diets containing a fixed amount of erucic acid was studied. If the level of dietary palmitic acid was increased the cases of liver cirrhosis decreased (79 6). Increased erythropoiesis in the liver has been observed in ducklings and guinea pigs fed a diet containing rapeseed oil (78, 79 1).

Effect on spleen

Rapeseed oil in the diet has no effect on the spleen of rats (68, 8, 2, 4 5). However the spleen of ducklings and guinea-pigs reacts. Ducklings receiving 30 cal% rapeseed oil or more in the diet showed *atrophy of the* red pulp, lipidosis and increased erythropoiesis in the spleen (3). Hypertrophic spleen *with enlarged fat infiltrated red pulp and an* intensive erythropoiesis were observed in guinea-pigs after six weeks on a diet with 50 cal% *rapeseed oil (78).* The lesions in the spleen of ducklings and guinea-pigs were decreased after the addition of hydrogenated *palm oil or tallow to the diet (79 6, 1).*

Effect on kidneys

After sixteen weeks or more on rapeseed oil diet rats showed changes in the kidneys. These changes consist of increased kidney weight and nephrosis characterised by vacuolation of the tubular epithelium, tubular dilatation and focal connective tissue proliferation. These changes increased severely with time (2, 4, 5). In female rats the renal concentrating capacity was lowered after intake of rapeseed oil for nine and twenty weeks (25).

Effect on blood

Rapeseed oil in the diet has no effect on the blood cholesterol level in rats, mice and

dogs, whereas a tendency to hypercholesterolemia is seen in guinea-pigs and chicks (34). A low level of rapeseed oil in the diet (6—8 %) did not influence the blood cholesterol in rabbits (88, 59). The results of experiments on rabbits with higher amounts of rapeseed oil in the diet are contradictory (34 5).

Dietary rapeseed oil increased the hematocrit and reticulocyt count in ducklings (3). Dietary rapeseed oil induced hemolytic anemia in guinea-pigs. If the diet contained isocaloric fat mixtures and equal amounts of erucic acid but increasing levels of palmitic acid, the values for hemoglobin content, packed cell volume and non-electrolyte hemolysis were improved with increasing amount of palmitic acid (79).

Effect on heart

The pathological effects on the myocardium of growing rats induced by feeding rapeseed oil have been distinguished into three stages: intracellular lipidosis, histiocytic infiltration and finally fibrosis (2, 4 5).

The intracellular myocardial lipidosis in rat starts some hours after supplying oil and reaches a maximum after 3—6 days thereafter decreasing rapidly (4 55 _1). Abdellatif and Vles (7) fed rats on a diet containing 0, 5 10, 15 20 _5 or 30 cal% European rapeseed oil (44 % erucic acid) for three or six days. The diets were made isocaloric in fat (40 cal%) by the addition of sunflowerseed oil. All rats fed rapeseed oil for three or six days showed a dose-related lipidosis of the heart. Beare-Rogers et al (71) fed rats 0, _5 5 10, 15 or 20 % Canadian rapeseed oil (33 % erucic acid) in a 20 % fat diet for one week. Pathological findings showed that 5 % rapeseed oil (10 cal%) had no effect and demonstrated some abnormal fat accumulation with 10 % (20 cal%) rapeseed oil in the diet. According to Engfeldt and Brunius (49) about 2 % erucic acid in the diet did induce pathological fatty accumulation in the heart muscle cells of rats, while 1 % did not.

The lipidosis of the myocardium never disappears completely (4 55 _1). Adult rats get a milder lipidosis than young ones (4 19 24).

Lipidosis of the heart muscle caused by feeding rapeseed oil, has been observed not only in rats but also in ducklings, guinea-pigs, rabbits, gerbils, miniature pigs, piglets and squirrel monkeys (3 79 5 _4). Furthermore rapeseed oil cause a severe hydropericardium in ducklings (3). The addition of palmitic acid to a diet rich in erucic acid decreased the hydropericardium incidence but did not improve the changes of the myocardium (6, 79).

Focal or diffuse infiltrations of mononuclear cells, histiocytes and proliferation of fibroblasts appeared in the myocardium of rats after four and eight weeks on rapeseed oil diet. These changes increased in severity and became less cellular and more fibrotic in the course of time (4). Prolonged feeding of rapeseed oil rather than a previous history of lipidosis seems to underlie fibrosis (19).

There are many indications that long chain monoenoic fatty acids, and especially erucic acid, are responsible for the pathological changes induced by rapeseed oil (2, 3 4 7). Hydrogenated herring oil, containing a high level of docosenoic acid (C 22:1), induces lipidosis and later degenerative lesions in the myocardium of rats (21 23). After one week a high intake of eicosenoate (C 20:1) produced myocardial fat droplets in rats, whereas erucate (C 22:1 Δ 13) or cetoleate (C 22:1 Δ 11) caused an appreciably greater accumulation of myocardial lipids (22).

Experiments have also been performed with canbra oil (about 2 % erucic acid and about _ % eicosenoic acid) on rats. Rocquelin et al (64 66) have observed changes, which they interpreted as myocarditis in rats after two and six months respectively on a diet containing 30 cal% canbra oil. The lesions were less frequent and severe in the group receiving canbra oil than in the one fed rapeseed oil. The six month experiment on rats with canbra oil diet was repeated by Abdellatif and Vles (4), no pathological

changes induced by canbra oil could be seen in this experiment. Feeding rats on a diet containing 60 cal% canbra oil for two weeks or a diet containing 50 cal% canbra oil for three days did not induce any pathological changes. Beare Rogers et 1 (21) for one week fed rats 10 or 20 % canbra oil in a 20 % fat diet, no abnormal fat accumulation could be seen in the heart.

The fatty accumulation in the heart muscle induced by dietary rapeseed oil causes a decrease in the capacity of isolated heart mitochondria to oxidize substrates (55). The findings suggest that a mitochondrial metabolite of erucic acid inhibits the mitochondrial oxidation of other fatty acids, especially in the heart, and that this causes the accumulation of triglycerides in the hearts of rats fed rapeseed oil (40, 41 51 50).

Engfeldt (personal communication) has examined hearts from 97 consecutive post mortem at Sabbatsbergs Hospital in Stock holm. In twelve cases fatty droplets could be observed in small areas of the fibres of the left ventricular muscle. The fatty infiltration had the same character and extension as in studies on rats fed rapeseed oil. The diagnosis for the 97 cases varied and the verage age was high. Nothing was known about food habits.

Effect on skeletal muscles

Rapeseed oil in the diet causes a reversible fatty infiltration in the skeletal muscles of rats. The skeletal muscles are pale after two weeks on diet with rapeseed oil (60 cal%) and have normal colour after eight weeks. After sixteen weeks on the diet the morphology of the skeletal muscles is normal (2, 4 5). Three weeks on a rapeseed oil diet (30 cal% or more) caused fatty accumulation frequently associated with edema and disintegration of the muscle fibres and, in some cases, with cell infiltration in the skeletal muscles of ducklings (3). These changes did not disappear when the rapeseed oil diet contained saturated fat (79 6, 1).

Effect of different fats on longevity

Male rats fed a diet containing 50 cal% rapeseed oil ad libitum lived 20 to 25 % longer than those fed a corresponding butterfat containing diet. Growth rate and daily food intake were lower for the group receivi g rapeseed oil diet. Histological examination gave no clear information about the cause of death (74). A later experiment with different amounts of rapeseed oil or butterfat in the diet justified the conclusion, that on feeding rapeseed oil the life-span of rats is not shortened (78). Thomasson et al (78) also studied the effect of different oils on the life-span of rats. The mortality in the groups fed maize oil, soybean oil, coconut oil, whale oil or rapeseed oil was practically the same, but the butterfat group displayed a shorter life-span. Also in experiments with mice butterfat diet caused shorter life-span than rapeseed oil diet (78).

Measures taken

From the nutritional point it is desirable to reduce or eliminate docosenoic acid from the diet. In Sweden rapeseed oil is consumed mainly as one of several fats in margarine. Hydrogenated marine oils are also used in some types of margarine. In 1970 the Association of the Swedish Margarine Manufacturers decided, that margarine produced in Sweden should contain maximum 15 % of the fat as rapeseed oil. Since 1972 it is made a rule that margarine may contain at highest 5 % docosenoic acid calculated on the total fatty acids. In Sweden erucic acid constituted 3—4 % of the average intake of calories up to 1970 compared with about 0.4 % at present.

Canada has announced that docosenoic acid may not exceed 5 % of the total fatty acids in the following provisions produced after 1973-11 30: margarine and margarine like products, shortenings, salad oils, coo ng oils, salad dressings and

This limitation can be met by using new low erucic acid varieties of rapeseed or by restricting the percentage of other sources of docosenoic acid, such as marine oils.

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Morphological Effects of Rapeseed Oil in Rats

I Short term studies

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Abstract

Light microscopy of paraffin embedded and frozen sections, supplemented with electron microscopy was performed on the heart muscle of young rats fed rapeseed oil in short term experiments It was confirmed that high levels of rapeseed oil, which contains erucic acid, produce severe lipoidosis of the heart muscle fibres within 10 days.

An attempt was made to find out the lowest level of erucic acid in the rat diet to give rise to pathological fatty accumulation. Several frozen sections from each heart or serial sections in combination with electron microscopy were used for this evaluation. The level found to give rise to pathological fatty accumulation was about 2 % by weight (w/w), while rats fed 1 % erucic acid showed normal myocardium.

No direct proof that erucic acid is of importance in human pathophysiology has hitherto been presented. It is concluded, however that the similarity in reaction among the many species of experimental animals tested by different workers, as well as the basic metabolic disturbances demonstrated, are in strong favour of a similar effect in man.

Introduction

Oil from the seed of different Brassica varieties has been used for many years in human food. It is the most important vegetable oil produced in Sweden. This particular oil is characterized by its content of long carbon chained fatty acids, especially erucic acid (C 22:1), which may account for as much as 50 % of the fatty acids of the oil.

In the late 1950s adverse effects of feeding laboratory animals with high doses of rapeseed oil were reported. Thus Roine et al. (1960) published results indicating the occurrence of myocarditis in rats fed 50 and 70 cal% of rapeseed oil. Similar experimental studies were repeated by several research workers in the late 1960s (2, 4 24 25 26). In these investigations it was established that young rats fed a diet with a high content of rapeseed oil show a rapid increase in the content of triglycerides in the striated muscle, including the myocardium. This so called lipoidosis reaches its maximum after about one week. After another one to two weeks it starts to decline and is reported to disappear after about two months.

Apart from the lipoidosis of the myocardium in young rats fed high doses of rapeseed oil, other alterations have also been observed after a period of two to four months, viz. myolysis, infiltration of the myocardium by histiocytes, and finally scarring. In long-term experiments other organic changes have been found, e.g. cirrhosis of the liver and alterations of the bone marrow cells (5). These latter changes do not seem to be very pronounced, however. In the evaluation of the pathophysiological effects of rapeseed oil on the organism they are of minor interest as compared with the myocardial alterations.

Experiments with rapeseed oil feeding have not been performed on rats only Most laboratory animals have been tested and they all react in a similar way with lipoidosis of the myocardium followed later by myolysis, inflammatory reactions and scarring. The

sensitivities of different experimental animals seem to vary however and there are also sex and age variations, males being more sensitive and younger animals reacting more severely To our knowledge no direct evidence of adverse effects of rapeseed oil in human beings has been presented so far

There has been much discussion as to what substance or substances are responsible for the described organ changes. It now seems to be generally agreed, however that erucic acid and its homologues are the main or sole causative factor

In Sweden the consumption of fat by human beings is rather high, approaching 40 % of the energy intake. Of the vegetable oils used in human nutrition rapeseed oil has been of considerable significance. Thus some of our margarines have contained up to 35 % w/w of this oil. The Swedish variety of rapeseed oil has a high content of erucic acid, reaching approximately 50 % w/w

The present investigation was undertaken two steps. In the first step presented here, ort-term experiments were performed on rats in order to varify the described early effects of feeding large amounts of rapeseed oil, in particular lipoidosis of the myocardium. An attempt was also made to find the limit below which no adverse effect could be induced in short term feeding experiments. As it seems established that it is the erucic acid content of rapeseed oil that determines the occurrence of pathological effects, this parameter was used in the attempt to find this limit.

Experimental

Altogether four series of experiments were carried out. These were designated series 1a, 2a, 3 and 11 The purpose of the first series was to find out whether the reported fatty accumulation in the myocardium could be reproduced in our laboratories. The other three series were undertaken in an attempt to ascertain the level of erucic acid in the food below which no lipoidosis of the myocardium could be induced.

Sprague Dawley rats were used for the experiments. In the first three series rats of a conventional breed and in the fourth, rats of a SPF breed were used. The rats were delivered from the breeder (Anticimex Ltd.) to our animal house at the age of 28 days and were kept there under conventional conditions (no SPF precautions). The feeding experiments lasted 10 days. During this period all animals received diets containing 40 cal% of fat. The diets were composed of casein 20 g, vitamins 0.24 g[1] minerals 5 g[2] cellulose flour 1 g, fat 21 g, and saccharose to make up to 100 g. In the case of margarines (series 2a and 3) due allowance was made for their content of water

The fats of the different diets were analysed for fatty acids by gas-chromatography The values obtained are presented in Table 1

The control animals in these experiments were given arachis oil. The dietary content of erucic acid was varied by using conventional rapeseed oil containing about 50 % erucic acid[3] rapeseed oil improved by breeding (Swedish Svalöv-Sinus) and containing about 10 % erucic acid, arachis oil-rapeseed oil mixtures or margarine. Each animal had its own wire cage provided with a raised bottom screen. Diet and tap water were given *ad lib* The animals were weighed immediately before they were put on the diet (age 28 days), *during the experiments, and* on the day of death

The first experimental series (1a) comprised 40 animals, 20 males and 20 females.

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1) Retinyl palmitate 0.0011 g, cholecalciferol 0.000005 g, dl-α-tocopherol acetate 0.005 g, dl-α-tocopherol 0.005 g, choline chloride 0.2 g, thiamine mononitrate 0.0005 g, riboflavin 0.0008 g, pyridoxine hydrochloride 0.0005 g, niacinamide 0.004 g, calcium pantothenate 0.004 g, p-aminobenzoic acid 0.01 g, bioti 0.00004 g, pteroylglumatic acid 0.0002 g, cyanocobalamin 0.000003 g, inositol 0.01 g and menadione 0.0005 g.

2) NaCl 0.7 g, KH_2PO_4 1.95 g, $MgSO_4 \cdot 7H_2O$ (in series 11 anhydrous $MgSO_4$) 0.29 g, $CaCO_3$ 1.91 g, $FeSO_4 \cdot 7H_2O$ 0.14 g, $MnSO_4 \cdot H_2O$ 0.02 g, $ZnSO_4 \cdot 7H_2O$ 0.0027 g, $CuSO_4 \cdot 5H_2O$ 0.0074 g, $CoCl_2 \cdot 6H_2O$ 0.00012 g, and KJ 0.0040 g (mineral mixture according to AOAC X and XI).

3) In this work percentage refers to weight % unless otherwise stated.

TABLE 1

Fatty acid contents of fats used in the different diets as determined by gas-chromatography
A = arachis oil, R = rapeseed oil, IR = improved rapeseed oil (Sinus), M = margarine, MR = margarine containing rapeseed oil

Diet No.	Fat	g fatty acid per 100 g of fat 12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	20:2	22:0	22:1	22	24:0
447 452, 458, 459	A	—	—	10.8	0.1	3.2	36.4	35.0	0.8	1.5	1.1	—	2.9	1.[illegible]	—	1.2
474 475 476	A	—	—	11.2	—	3.1	34.2	38.1	0.8	1.1	1.4	—	2.9	0.4	—	1.0
448 453	R	—	—	3.0	0.2	0.8	10.5	13.2	8.6	0.3	6.9	0.4	0.3	49.2	0.6	0.8
456	R	0.2	0.1	3.3	0.2	1.0	11.8	13.5	8.9	0.5	8.2	0.4	0.7	46.6	0.9	0.8
475, 476	R	—	—	[illegible]	0.1	0.7	11.6	12.8	8.4	0.4	7.9	0.6	0.2	49.2	0.2	0.5
451 457 458	IR	—	0.1	4.3	0.3	1.1	40.9	19.4	10.4	0.4	6.0	—	0.2	11.2	—	—
449	M	10.4	4.8	15.9	0.5	5.9	27.1	26.6	0.6	0.2	0.2	—	0.2	0.5	—	—
454	M	10.9	4.6	9.4	0.2	6.5	28.9	30.5	0.9	0.2	0.1	—	0.4	0.4	—	—
450	MR	9.0	6.5	13.0	2.8	6.0	20.9	15.2	2.6	1.3	4.1	0.9	1.0	10.0	—	—
455	MR	6.7	6.1	14.3	3.2	6.6	21.8	15.1	2.7	1.2	4.8	1.1	0.7	9.0	—	—

TABLE 2

Degree of myocardial lipoidosis of Sprague Dawley rats receiving diet with 40 cal% of fat and different contents of erucic acid (C 22:1). Experimental time 10 days

Exp series	Number of animals	Diet No.	Fat component of diet	C 22:1 % w/w in diet	Degree of lipoidosis
1a	10 ♂ + 10 ♀	447	Arachis oil	0.3	0
	10 ♂ + 10 ♀	448	Rapeseed oil	10.3	3+
2a	10 ♂	449	Margarine	0.1	0
	10 ♂	450	Margarine containing rapeseed oil	[illegible]	1+
	10 ♂	451	Improved rapeseed oil	2.4	1+
	10 ♂	452	Arachis oil	0.3	0
	10 ♂	453	Rapeseed oil	10.3	3+
3	10 ♂	454	Margarine	0.1	0
	10 ♂	455	Margarine containing rapeseed oil	1.9	1+
	10 ♂	456	Rapeseed oil	9.8	3+
	10 ♂	457	Improved rapeseed oil	2.4	1+
	10 ♂	458	Arachis oil + refined rapeseed oil (1+1)	1.3	0
	10 ♂	459	Arachis oil	0.3	0
11	5 ♂ + 5 ♀	474	Arachis oil	0.1	0
	5 ♂ + 5 ♀	475	Arachis oil + rapeseed oil (1+0.[illegible]4)	2.1	1+
	5 ♂ + 5 ♀	476	Arachis oil + rapeseed oil (1+30.3)	10.0	3+



Fig 1 a. Photomicrograph of formalin-fixed frozen section from heart muscle of rat fed 40 cal% rapeseed oil 10—15 μ thick section stained with Scharlach Rot. Fat droplets of varying size arranged along the fibres are observed. x 220

Half of these animals, 10 of each sex, were fed a control diet containing arachis oil. The other half were fed a rapeseed oil diet, containing about 10 % erucic acid.

The second and third experimental series (2a and 3) comprised five and six groups, respectively with 10 animals in each group thus totalling 110 animals. The fourth series (11) comprised 30 animals. In these last three series the levels of erucic acid in the diet varied from 10 % as used in series 1 down to 0.3—0.1 % in the control groups. In series 2a and 3 two experimental groups were added these received the 40 cal% of fat in the form of two types of conventional margarines with and without rapeseed oil. The different experimental series and the calculated content of erucic acid in the diets are presented in Table

After 10 days on these diets the abdomen was opened under light ether anaesthesia and the animal was killed by exsanguination. The liver, kidney, spleen and heart were immediately removed and weighed.

A sagittal control slice about 1 mm thick was cut from the heart and from this slice small pieces measuring about 1 mm were dissected and were fixed in either glutaraldehyde or osmium tetroxide. Five to six pieces were fixed in each fixative. The remainder of the tissue slice was fixed in glutaraldehyde and thereafter stored in cacodylate buffer at +4 C for future use. The rest of the heart tissue, which comprised two pieces, was fixed in neutral formalin. The right part of the heart was taken for fat staining, using a cryostat (Leitz) for sectioning. Two to three sections from each

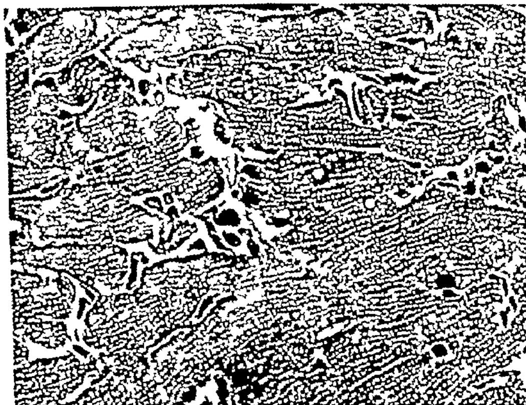


Fig 1 b Photomicrograph of glutaraldehyde fixed epon-embedded section *from heart muscle of rat fed 40 cal% rapeseed oil.* μ thick section. Phase contrast. Vacuoles of varying size are observed in each muscle fibre x 260

block were stained with Scharlach Rot. In certain cases in the low erucic acid groups serial frozen sections were cut. The left part of the heart was embedded in paraffin and 3—5 μ thick sections were cut on a microtome and were stained with Ehrlich's acid hematoxylineosin. In certain cases serial sectioning was performed, every 10th section being taken. The pieces for electron microscopy were taken through graded alcohols and then embedded in epon. After rough trimming 1—2 μ thick sections were cut on the ultratome for phase contrast studies. From these observations small areas were selected for further ultrathin sectioning. These sections were contrast-stained with uranyl acetate and lead citrate and the material was examined in a Siemens Elmiscope I

The lipoidosis of the heart muscle was evaluated in a light microscope on Scharlach Rot stained frozen sections. The amount of lipid droplets deposited in the myocardium was graded as follows: 0 = no lipid droplets 1+ = a few areas containing small lipid droplets; 2+ = lipid droplets in 5—25 % of the muscle fibres; 3+ = lipid droplets in 25—100 % of the muscle fibres.

This light microscopic evaluation of the amount of lipoidosis in the myocardium was supplemented by electron microscopic observations of material from the different experimental groups receiving 0.1—10 % erucic acid.

Specimens of the liver spleen, kidney and striated muscle from rats of series 1a and 2a were processed for light microscopic examination.



ig 2. Photomicrograph of glutaraldehyde-fixed epon-embedded section from heart muscle of rat fed 40 cal% margarine MR 2 μ thick section. Phase contrast. In an area in the middle small vacuoles are seen in the muscle fibre x 300

Results

In none of the series were any significant differences in weight increase observed between the control animals receiving arachis oil and those receiving the other fats.

An outline of the observations on myocardial lipoidosis made in the different series is presented in Table 2. In the first series on 40 animals, covering 10 days, pronounced (3+) lipoidosis of the myocardium was found in all animals receiving rapeseed oil (Fig 1a), and at autopsy the heart was found to be pale creamy yellow in colour The control animals fed arachis oil showed no fat droplets on light microscopy The fat droplets of the rapeseed oil rats were located in the muscle fibres. They were round or oval, varied up to 5 μ in diameter and were arranged in rows along the longitudinal axis of the fibres (Fig 1b). Apart from the lipoidosis of the myocardium and skeletal muscles no other organic changes were observed in this experimental series.

In the following three series of experiments similar changes were seen in the hearts of rats receiving 10 % of erucic acid in the food, while those fed arachis oil exhibited no myocardial lipoidosis.

In the groups of rats given about 2 % of erucic acid in the food, the myocardium showed areas with small fat droplets arranged in rows along the longitudinal axis of the muscle fibres (Fig 2) Sometimes these droplets were hardly discernible when ordinary transmitted light was used These groups of rats were given both improved rapeseed oil (Span) and margarine con

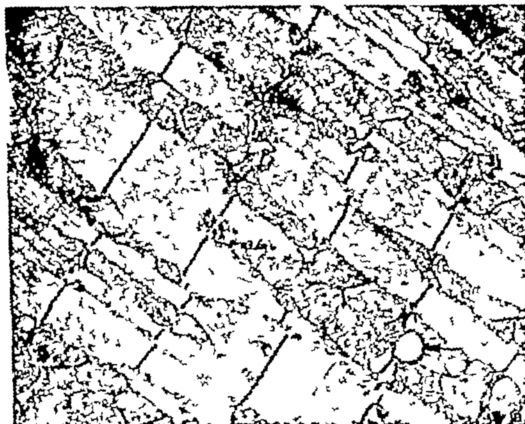


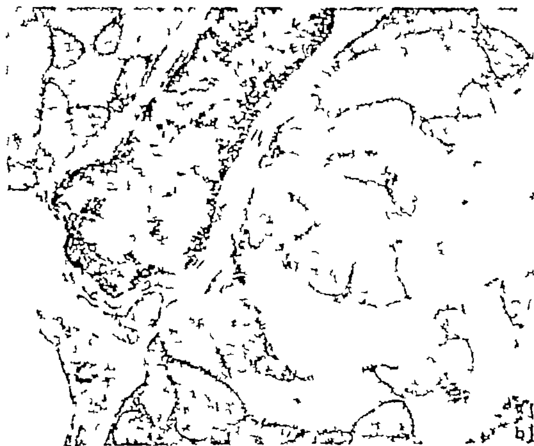
Fig 3 a Electron micrograph of glutaraldehyde-fixed epon-embedded ultrathin section from heart muscle of control rat fed 40 cal% arachis oil. Longitudinal section. Normal fine structure: note however that one lipid droplet in close contact with the mitochondria is found in the lower part of the picture x 11,000

taining rapeseed oil. In rats given food containing about 1 % of erucic acid or lower no lipoidosis was found on light microscopy

On electron microscopic examination, however fat droplets were seen in some locations even at the low erucic acid level of 1 % but also in a few places in the control animals fed with arachis oil (cf. Figs 3a and b and 4). In the myocardium of the rats given 10 % of erucic acid almost every muscle fibre contained fat, and the droplets were generally located in close contact with the mitochondria. In sections cut longitudinally to the muscle fibres the droplets were fairly often located in areas corresponding to the Z lines. The fat globules were spherical and fairly electron dense, in both osmium and glutaraldehyde fixed specimens. No definite indication of a surrounding membrane was observed. Sometimes the lipid droplets left impressions on the mitochondria (Fig 5), but otherwise the cell organelles showed no characteristic alterations, and no other structural effects on the heart muscle were observed. No reaction of the interstitial compartment was found in these experiments.

Discussion

In this study we were able to confirm earlier reports on the effect on the myocardium



g 3 b Electron micrograph of glutaraldehyde fixed epon-embedded ultrathin section from heart muscle of control rat fed 40 cal% arachis oil. Trans erse section. Normal f ne structure. x 18,000.

of short-term feeding with diets containing high contents of rapeseed oil. It has been found by Beare-Rogers et al., Abdellatif and Vles, Rocquelin and Churan and Bodak that after a few days on diets rich in rapeseed oil experimental animals exhibit fatty accumulation in the heart muscle fibres. This reaches its maximum after 5—10 days and thereafter declines.

Accumulation of lipid droplets in the heart muscle f bres is observed in several pathological conditions, such as stress, hypoxia and intoxications (29 20, 15 16, 21). It is thus in no way a specific finding. The mechanism underlying lipoidosis of the heart muscle cell after rapeseed oil feeding has been much discussed and there is now general agreement that it is the long-chain fatty acids, above all erucic acid, that are the sole or major cause of this condition. Evidence has recently been presented that erucic acid, which is the major fatty acid of rapeseed oil, has a reducing effect on the β-oxidation of fatty acids of cardiac muscle cells *(18). This effect may conceivably result* in an accumulation of lipids outside the mitochondria. For details on this point the reader is referred to the paper by Heijkenskjöld and Ernster in this volume.

It has thus been established that feeding diets containing high amounts of long-chain fatty acids to rats gives rise to pathological alterations, above all lipoidosis of the heart muscle fibres. This particular change has been observed also in several other species, such as rabbits, pigs, squirrel-monkeys, gul-



Fig 4 Electron micrograph of glutaraldehyde-fixed epon-embedded section from heart muscle of rat fed 40 cal% improved rapeseed oil In this longitudinal section several lipid droplets are observed, most of them located inbetween the mitochondria. x 33.000

nea pigs, dogs, chicken and ducklings (28 2, 3 11) N direct observations ha e been re ported as to the effect of these fatty acids on human cardiac muscle It is known, how ever that the above described type of fatty accumulation in the myocardium has also been observed under certain conditions in human pathology (2.) It thus seems very probable that the human heart will react similarly to the heart of experimental animals when exposed to certain levels of long-chain fatty acids. From this premise it seems of importance to look for the limit of these fatty acids in the food below which no lipidosis of the heart muscle can be observed. A few incomplete and controversial data on this question have hitherto been published. Abdellatif and Vles (1970) (2) performed dose-response studies over two weeks and found that 70 cal% European rapeseed oil containing 46 % erucic acid was the minimum level to cause fatty accumulation this means a level of about 4.5 % erucic acid in the diet. In similar studies

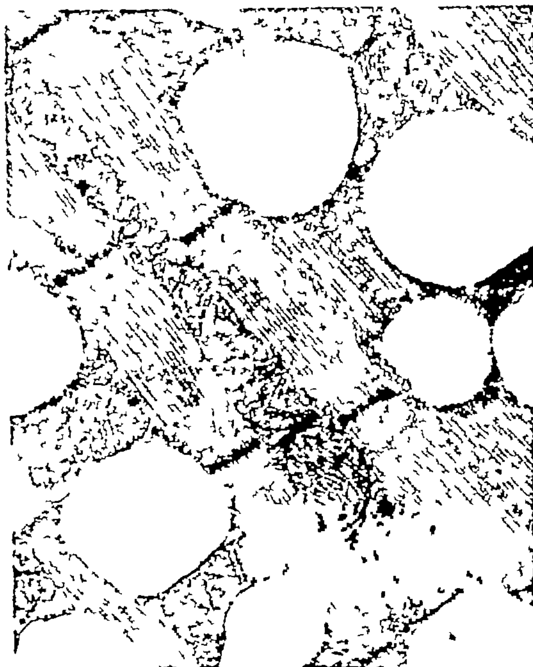


Fig 5 Electron micrograph of longitudinal section of glutaraldehyde-fixed epon-embedded section from heart muscle of rat fed 40 cal% rapeseed oil. Many lipid droplets of varying size are found among the mitochondria. They do not seem to be enclosed by a membrane. The mitochondria in contact with the droplets show irregular impressions. x _8,000

Beare-Rogers (1970) (7) found the "zero-effect" level to be 10 % by weight. In this experiment a Canadian rapeseed oil containing 33 % erucic acid was used, which corresponds to a level of about 3 % erucic acid in the diet. In our experiments, studies of serial frozen sections revealed no pathological lipoidosis in rats given food containing 1 % erucic acid, while 2 % gave pathological alterations. It should be pointed out, however that occasional fat droplets were seen with the electron microscope even in our controls fed with arachis oil. Such findings are considered normal.

In many countries of the world long-chain fatty acids form a constant part of the fat consumed by humans. In our country most of these acids have come from rapeseed oil, which constitutes an ingredient of some of our margarines. Similar fatty acids can be traced to other sources, above all marine oils and fat-rich fish. The level of long-chain fatty acids in the food varies considerably according to different factors, including food habits. The 1 % level of erucic acid may be a reasonable no effect limit for humans also, and it seems probable that many people in our country have had an intake around this level (cf Borg this volume).

It is not possible to demonstrate conceivable pathophysiological effects of long-chain fatty acids by the experimental technique used in the present study which deals only with the morphological events. However the decrease in mitochondrial respiration observed in the heart muscle in the experiments by Heijkenskjöld and Ernster (this volume) indicates unequivocal pathophysiological alterations resulting from feeding erucic acid.

Acknowledgement

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Fig 5 Electron micrograph of longitudinal section of glutaraldehyde-fixed epon embedded section from heart muscle of rat fed 40 cal% rapeseed oil. Many lipid droplets of arying size are found among the mitochondria. They do not seem to be enclosed by a membrane. The mitochondria in contact w th the droplets show irregular impressions. x 28 000

Morphological Effects of Rapeseed Oil in Rats

II Long term studies

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Abstract

In long-term studies covering up to 160 days young Sprague Dawley rats were fed diets containing 40 cal% of fat The fat component consisted of either conventional rapeseed oil, or Canadian rapeseed oil low in erucic acid, or arachis oil Myocardial fatty accumulation was demonstrated in light microscopic studies throughout the experiments in rats fed conventional rapeseed oil, but the number of fat droplets decreased with time. The controls fed arachis oil showed no fatty accumulation In the rats fed conventional rapeseed oil focal myocardial lesions ppeared after 40 day on the diet. These consisted of histiocytic infiltration, occurrence of macrophages, myolysis, proliferation of f broblasts and finally scarring. Such foci were found widely spread in the myocard um of these rats. In the experimental groups given Canadian rapeseed oil from the cultivar Oro no histiocytic foci or scarring were observed. Small myocardial lesions were occasionally found in the control rats. These latter findings were observed on serial sections. It was concluded that this type of lesion is a normal" finding. The number and size of the foci observed in animals fed conventional rapeseed oil (10 % and % (w w) erucic acid in the diet) indicate however that they have to be considered pathological under such circumstances The pathogenesis of the myocardial lteration is discussed and it is concluded that the long-chain fatty acids are responsible No direct proof has been presented that the described events are of importance in human pathophysiology However several circumstances pointing in this direction are discussed. It is concluded that on the basis of our present knowledge a pathological effect of erucic acid and is homologues in man cannot be excluded

Introduction

It has been demonstrated by several investigators that rapeseed oil retards the growth of rats. It has also been established that erucic acid, which constitutes about 50 % of the fatty acids of rapeseed oil, is the substance responsible for the growth retardation (23). In spite of this finding pathological effects of feeding rapeseed oil to animals have only been sparsely investigated. In 1960 Roine et al. reported a study on the morphological effects of rapeseed oil fed to rats and pigs They found that on receiving a diet containing 50 cal % rapeseed oil these animals suffered alterations of the myocardium, which they designated myocarditis. Other organs studied showed no pathological changes. The myocardial effects described by Roine et al. ha e since been confirmed by several research groups (19 7 4 6, 12).

In short-term experiments in which high levels of rapeseed oil have been given to animals, myocardial changes have been found to occur rapidly After only one or two days on the diet young rats have exhibited fatty accumulation of the myocardium. This effect is reported to reach a peak in five to ten days and thereafter to decline. The fatty accumulation is also found in skeletal muscle, which reacts similarly to the heart.

TABLE 1

Fatty acid contents of fats used in the different diets, as determined by gas chromatography
A = arachis oil, R = rapeseed oil, CR = Canadian rapeseed oil (Oro)

Diet No.	Oil	g fatty acid per 100 g of oil													
		14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	20:2	22:0	22:1	22:2	24:0
453	R	—	3.0	0.2	0.8	10.5	13.2	8.6	0.3	6.9	0.4	0.3	49.2	0.6	0.8
462	A	—	10.8	0.1	3.2	36.4	35.0	0.8	1.5	1.1	—	[illegible].9	1.2	—	1.2
463	R	0.1	3.3	0.[illegible]	1.0	11.8	13.5	8.9	0.5	8.[illegible]	0.4	0.7	46.6	0.9	0.8
474 475 476	A	—	11.2	—	3.1	34.2	38.1	0.8	1.1	1.4	—	2.9	0.4	—	1.0
475 476	R	—	2.8	0.1	0.7	11.6	12.8	8.4	0.4	7.9	0.6	0.2	49.[illegible]	0.2	0.5
477	A	—	11.3	—	3.0	38.7	35.1	2.[illegible]	—	1.0	—	2.8	0.1	—	0.9
478	CR	0.1	4.3	0.2	1.4	59.9	17.1	8.3	0.5	1.1	—	0.2	0.3	—	—
479[1]	R_1	—	2.8	0.1	0.7	11.6	12.8	8.4	0.4	7.9	0.6	0.2	49.2	0.2	0.5
	R_2	0.7	2.3	—	1.0	16.6	14.8	10.3	—	8.6	—	0.6	40.1	0.5	—

[1]) Rapeseed oil R_1 was used up in the course of the experiment and replaced by R_2.
cf. Table 2, series 12, diet 479

TABLE 2

Designs of the experimental series and computed erucic acid contents of the diets

Exp. series	Number of animals		Diet No.	Fat component of diet	C 22.1 % w/w in the diet	Exp. time days
2 b	20 ♂		453	Rapeseed oil	10.3	18, 40, 80, 160
5	10 ♂	5 ♀	462	Arachis oil	0.3	88, 139
	10 ♂	5 ♀	463	Rapeseed oil	9.8	88, 139
11	20 ♂	20 ♀	474	Arachis oil	0.08	10, 20, 40, 80
	20 ♂	20 ♀	475	Arachis oil+rapeseed oil (1+0.24)	[illegible].1	10, 20, 40, 80
	20 ♂	20 ♀	476	Arachis oil+rapeseed oil (1+30.3)	10.0	10, 20, 40, 80
	15 ♂	15 ♀	477	Arachis oil	0.02	40, 80, 160
	15 ♂	15 ♀	478	Canadian rapeseed oil	0.06	40, 80, 160
	15 ♂	15 ♀	479	Rapeseed oil[1])	10.3 9.6 9.8	40 80 160

[1]) cf. note under Table 1

In the heart, however, additional changes result from diets with high levels of rapeseed oil. The lesions observed after a few weeks have been described as myolysis, necrosis and histiocytosis and those seen after a few months as incipient fibrosis. After still longer periods scarring of the myocardium has been found. There has been some controversy as to what substance or substances in the rapeseed oil provoke these changes. There seems to be general agreement that erucic acid and its homologues are the chief responsible components. However there are also reports indicating that rapeseed oil improved by breeding and containing low levels of erucic acid also gives rise to myocardial lesions (20).

Changes in other organs apart from the myocardium have also been reported, including nephrosis of the kidney, cirrhotic changes of the liver, hyperplasia of the adrenal cortex, and atrophy of the testis. Haematologic changes with development of haemolytic anaemia have also been described (2).

The aim of the present investigation was to elucidate the morphological alterations occurring in long-term experiments with rapeseed oil given to young growing rats, with special attention to cardiac lesions.

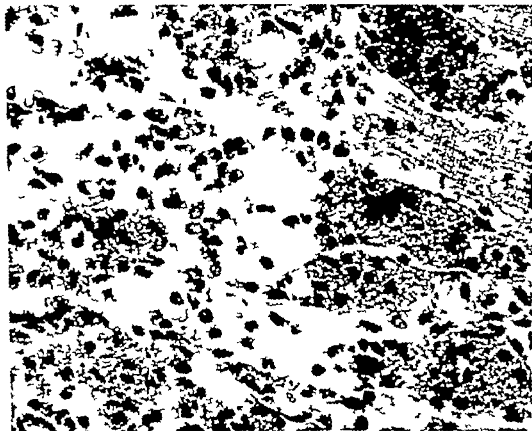


Fig 1 Photomicrograph of 10 μ thick frozen section of myocardium from a male rat fed 40 cal% rapeseed oil for 88 days Note the muscle fibres loaded with lipid droplets in the right part of the figure. In the left part the fibres have disappeared and are replaced by histiocytes, lymphocytes and macrophages containing lipid droplets. Staining, Scharlach Rot. x 350

Experimental

Four series of experiments, designated a b, 5 11 and 12, were performed, using Sprague Dawley rats as the test animals. The rats were supplied from an SPF breed, except those of the first series, which came from a conventional breed. They were all delivered from the dealer (Anticimex Ltd.) to our animal house at the age of 8 days and were maintained there under conventional conditions (no SPF arrangements).

All animals were given diets containing 40 cal% of fat. The diets were composed of casein 20 g, vitamins 0.24 g[1] minerals 5 g[1] cellulose flour 1 g, fat 21 g and saccharose to make up to 100 g.

The fats contained in the different diets were analysed for fatty acids by gas chromatography The results of the analyses are summarized in Table 1

Control animals were given arachis oil. The content of erucic acid in the diets was varied by using (1) conventional rapeseed oil containing 40 to 50 % erucic acid[2] (2)

[1]) See footnotes on p. 16 of our foregoing paper Anhydrous $MgSO_4$ was used in series 11 and 12, $MgSO_4$ $7H_2O$ in the other series.

[2]) In this work percentage refer to weight % unless otherwise stated.



ıg 2 Photomicrograph from another area of the same preparation as in Fig 1 showing a lesion with many lipid-loaded macrophages (arrow) x _50

arachis oil-rapeseed oil mixtures and (3) rapeseed oil from the Canadian cultivar Oro (ORO-FRI 7? 1 RKD (33)) containing only 0.3 ⌐ erucic acid the latter oil was kindly placed at our disposal by the Food Research Institute of the Canada Department of Agriculture. The designs of the different series and the computed content of erucic acid in the diets are presented in Table 2.

Diet and tap water were supplied *ad lib.* Each animal was kept in a separate wire cage provided with a *raised bottom screen.* The animals were weighed before being placed on the diet (age _8 days) during the experiment, and on its termination. The feeding experiments covered up to 160 days Altogether _60 animals were used in the four series.

For light microscopy the material from the heart, kidney liver and adrenals was treated in the same way as described in the foregoing paper except that the microtome sections were stained with Weigert s iron haematoxylin and Hansen s stain. Serial sec tioning of the whole heart was performed on paraffin-embedded blocks in all animals of series 1 and the 80-day group of series 11 In the last of the four series material was taken for electron microscopy For this, two animals from each group of 10 under went perfusion via the abdominal aorta, under ether anesthesia and with a pressure of 100 mm Hg first 20 ml of 0.4 % xylocaine in Tyrode s solution were gi en, followed by ⌐ glutaraldehyde in 0 1 M cacodylate buffer containing 0 1 M sucrose.



Fig 3 Photomicrograph of 5 μ thick paraffin-embedded microtome section showing myocardial lesion from a rat fed conventional rapeseed oil for 160 days. Close to the pericardial surface an area is observed where the muscle fibres have disappeared and are being replaced by a loose scar tissue where fibroblasts and collagenous tissue are inter mingled with lymphocytes and histiocytes.
Staining with Weigert's iron haematoxylin and Hansen's stain. x 200.

Altogether 250 ml were infused. The heart was then opened and the papillary muscle was removed and fixed as described in the previous paper Small pieces from the liver and the kidney were also taken for further electron microscopy

Results

In these long-term studies growth retardation was noted in the animals given conventional rapeseed oil, this finding is in accordance with observations of previous workers. Animals receiving the Canadian rapeseed oil did not show any growth retardation. No casualties occurred among the animals.

Light microscopy on frozen sections from the myocardium stained for fat revealed severe fatty accumulation after 10 days on a diet containing 40 cal% conventional rapeseed oil. Pathological alterations of the heart were also evident on gross examination at autopsy These were similar to the changes observed in our previous study where animals were fed for 10 days with a similar diet. The fatty accumulation within the heart muscle cells was somewhat less pronounced in experiments lasting 40 days. In animals fed a conventional rapeseed oil diet for 80 and 160 days the fatty accumulation showed a clear tendency to decline. The myocardial lipoidosis seemed to be more marked in the male rats. No lipoidosis of the heart was observed in light microscopic studies of animals given the control diet consisting of 40 cal% arachis oil for 10, 20 40, 80 or 160 days. Nor could any lipoidosis of the heart be demonstrated in animals after 40 80 or 160 days on the diet containing the improv-

Fig 4 Photomicrograph of 5 μ thick paraffin-embedded microtome section showing part of a myocardial lesion from a rat fed conventional rapeseed oil for 80 days. A large area with muscle fibres replaced by lymphocytes and histiocytes is observed.
Staining with Weigert's iron haematoxylin and Hansen's stain. x 220

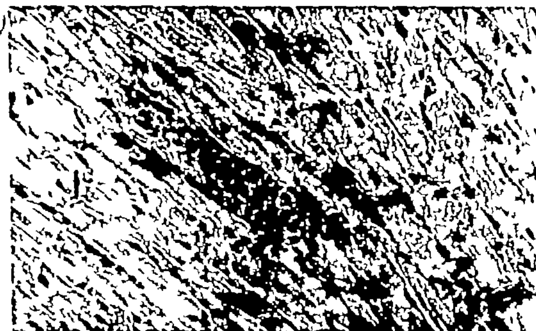


Fig 5 Photomicrograph of 5 μ thick paraffin-embedded microtome section showing a small accumulation of histiocytes in the myocardium of rat fed arachis oil 40 cal% for 80 days
Staining with Weigert's iron haematoxylin and Hansen's stain. x 280

ed Canadian rapeseed oil, i.e. the diet with only 0.06 % erucic acid.

The animals fed 40 cal% conventional rapeseed oil for 40 days also exhibited other heart lesions. Numerous small foci of histiocytes were seen in-between the muscle fibres, and macrophages containing lipid droplets were also sometimes demonstrated (Fig 1 and 2). These lesions were found in all experimental animals on this diet. In the male rats, however the foci were larger and more frequent. These lesions seem to have a preferential localization close to the pericardial surface (Fig 3). Similar lesions were also found in the experiments with 40 cal% rapeseed oil given for 80 and 160 days. In these instances, however the histiocytes were also mixed with varying numbers of fibroblasts and a collagenous scar tissue was finally produced (Fig 3). In the latter groups some areas with confluent lesions were observed and quite large portions of the myocardial wall were sometimes involved (Fig 4). The number of heart muscle fibres replaced by scar tissue was definitely larger in the male rat.

On going through the serial sectioned material of series 11 we found that rats fed on diet 475 (2.1 % erucic acid) for 80 days showed similar lesions to those described above. The foci were, however more sparse. Very small histiocytic foci were also found (Fig 5) occasionally in the control groups (diet 474), but these foci were considerably less frequent than in the other groups reported. In the serial sectioned material from series 12, no myocardial alterations were observed either in the group receiving arachis oil (diet 47" with 0.02 % erucic acid) or in the group receiving Canadian rapeseed oil (diet 478 with 0.06 % erucic acid).

Electron microscopy of the heart muscle from animals of series 11 and 12 fed conventional rapeseed oil (10 % erucic acid in the diet) revealed lipoidosis of the same type as described in our foregoing paper The fat globules found in connection with the mitochondria were definitely more frequent in the 40-day experimental group than in the 160-day experiment (Fig 6 d, 7 and 8). When comparing the findings of the experimental group fed conventional rapeseed oil for 10 days with the 40, 80 and 160-day experiments the lipid droplets were found to be smaller and more uniform in size in the latter

In the 40-day group of series 11 receiving diet 475 (2.1 % erucic acid) there was still some numerical increase in lipid droplets as compared with the finding in the animals on arachis oil (Fig 6 c). Small lipid droplets were also observed occasionally in the heart muscle fibres of rats fed diet 475 for 80 days, as well as in the control groups at all time points (Fig 6 a). These latter findings were considered normal. Further studies on the fine structure of heart muscle from rats fed conventional rapeseed oil for 40 80 and 160 days revealed foci corresponding to the histiocytic infiltration seen in the light microscope. Replacement of muscle fibres by scar tissue was also observed in the later experimental periods in rats fed rapeseed oil. Examination of the fine structure of the muscle fibres surrounding these areas disclosed no specific alterations apart from the above described accumulation of lipid droplets occurring in close contact with the mitochondria. The different cell organelles all seemed to have a normal structure and the myofibrils of the heart muscle showed no pathological alteration.

Light microscopy of the kidney and the liver from animals in the present experiments did not reveal any specific changes. However in the 80 and 160-day groups fairly advanced nephrocalcinosis was found with deposition of small calcium phosphate globules in areas between the cortex and the medulla. On X-ray diffraction these globules exhibited the pattern of calcium hydroxyapatite of a fine crystalline type. Such changes were seen both in the control rats and in the rats fed rapeseed oil and were almost exclusively confined to the female animals. No noticeable difference was found between the experimental groups receiving diets containing different amounts of $MgSO_4$

Fig 6 a. (above)

Fig 6 b (below)



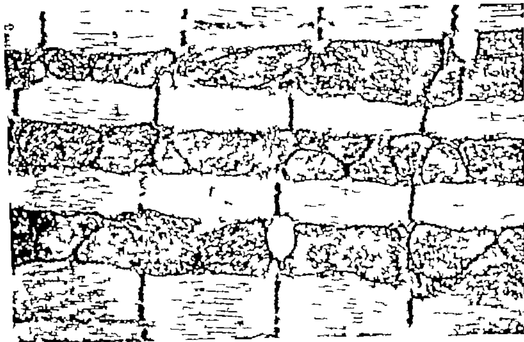


Fig 6 c. (above)

Fig 6 d. (below)



Fig 6. Electron micrograph of ultrathin section of myocardium from rats fed experimental diets for 40 days. Staining: uranyl acetate, lead citrate.

a) Longitudinal section from a control. x 13,000

b) Detail from the same specimen showing a megamitochondrion. 31,000

c) Longitudinal section from rat fed a diet containing 2 % erucic acid. x 16 000

d) Transverse section from a rat fed a diet containing 10 % erucic acid. x 10 000



Fig 7 Electron micrograph of ultrathin section of myocardium from a rat fed 40 cal% conventional rapeseed oil for 80 days. Transverse section showing an intercalated disk and a few lipid droplets. Staining uranyl acetate lead citrate x [illegible]6,000



Fig. 8. Electron micrograph of ultrathin section of myocardium from a rat fed 40 cal% conventional rapeseed oil for 160 days. Transverse section showing in the upper part a small blood vessel and bundles of collagen fibres. Staining: uranyl acetate, lead citrate x 12,000

Discussion

In our previous light microscopic studies we observed that fatty accumulation in the heart muscle cells occurred when experimental animals were fed rapeseed oil with a high content of erucic acid, i.e. above a level of about % in the diet. When 40 cal% of conventional rapeseed oil was given for 10 days (10 % erucic acid in the diet), heavy lipoidosis was evident and this could even be observed on gross examination. In the present study covering longer periods of time the lipoidosis resulting from a conventional rapeseed oil diet successively declined. It should be pointed out that the disappearance of the fat droplets from the myocardium in these long-term experiments seemed to be more definite in females. However pathological fatty accumulation in the heart muscle cells as revealed by serial frozen sections and electron microscopy could be demonstrated in both sexes even after 160 days on conventional rapeseed oil.

Light microscopy of the kidney and liver revealed no typical changes in these long-term experiments. However, in the kidneys of the female rats which were fed our diet for 80 and 160 days we found deposition of calcium phosphate: this observation was made both in controls and in animals fed rapeseed oil. This finding thus does not seem to have any connection with the feeding of rapeseed oil. Similar observations have been made by several research workers on animals having quite different diets (13). The nephrocalcinosis, which was most frequently found in the older female rats, was thus considered a non-specific finding.

Apart from the fatty accumulation of the myocardium other lesions have been reported in long-term feeding experiments in animals given rapeseed oil. After 4—8 weeks, when there is still considerable accu-

mulation of fat in the heart muscle cells, focal lesions have been found by various authors. The findings were necrosis, proliferation of mononuclear cells and the presence of histiocytes, leading to the development of scar tissue. Abdellatif and Vles (1971) have described the effect of dietary rapeseed oil in rats and rabbits. Their experiments were performed over 64 weeks. Similar reports have been published by Rome et al (1960) from studies on pigs and rats and by Beare Rogers et al. (1972) whose investigations on rats, squirrel monkeys, pigs and gerbils have included both chemical analyses of the myocardial fatty acids and histological studies. In our experiments we found that after 40 days on a diet with 10 % of erucic acid, foci with histiocytic infiltration appeared in the myocardium. After 80 days these lesions still showed numerous histiocytes intermingled with macrophages, containing fat droplets in the cytoplasm. At this time point we also found a considerable amount of collagen in the foci and in the 160-day experiment the number of histiocytes had decreased further while the fibroblasts and collagen had increased. The morphological events observed in our long-term experiments with rapeseed oil are thus in good accordance with the earlier reports by Abdellatif and Vles and Beare-Rogers and Nera. The above discussed focal lesions were pronounced in rats fed diets containing 10 % of erucic acid.

Controversial results from long-term feeding experiments with rapeseed oil containing very little erucic acid (so called canbra oil) have been reported in the literature. Rocquelin et al (1970) have described histiocytic infiltration, myocarditis in rats fed canbra oil for several months, while Abdellatif and Vles (1971) found no such lesions. In our experiments, series II the alteration observed in the myocardium of some of the controls and of the animals on diets containing % or 10 % of erucic acid did not differ qualitatively but in number and extent. The histiocytic infiltration occurring after 40 and 80 days on diet containing high levels of erucic acid was definitely pathological in character. At the % erucic acid level the individual heart lesion was of the same character as at the 10 % level but consistently smaller. Furthermore numerous foci were demonstrated in each heart on serial sectioning of the % erucic acid group compared with the controls, where only a few animals revealed scanty lesions. In our experimental groups (series I I) fed the Canadian rapeseed oil containing only 0.06 % erucic acid, no pathological lesions were observed and in the controls receiving arachis oil normal findings were made.

In a recent study Abdellatif and Vles (1973) report mononuclear cell infiltration and fibrotic scars occurring in the heart muscle of some experimental animals fed a diet containing 4 cal% erucic acid for 4 weeks. This finding is in close accordance with the results reported in this paper.

It seems conceivable from the above observations that small sparse foci of histiocytes in the myocardium might occur in conventionally fed rats (cf. Gaunt et al. 1967). This might be one reason for the controversial reports concerning the myocardial effect of "zero erucic acid" rapeseed oil. Furthermore the canbra oil used in the experiments of Rocquelin et al. (1970) contained not only 2 % erucic acid but also 2 % gadoleic and nervonic acid. The combined effect of the three acids might also have contributed to the pathological changes described.

There is nothing in our results to contradict the assumption that the pathological findings described in the myocardium are caused by erucic acid. It should be pointed out, however that Beare-Rogers (1970) has supplied evidence to the effect that herring oil rich in long-chain monounsaturated fatty acids (C 20:1 C 22:1) also produces cardiac lipoidosis in the rat (cf. also 15 and 16). Thus erucic acid and also other similar long chain fatty acids seem to be of pathogenetic importance.

The pathogenesis of the myocardial changes has only been slightly touched upon. The lipoidosis is the first event, followed by

myolysis and necrosis, histiocytic infiltration and finally scarring. There is no definite proof however that these different alterations are causally linked to one another. The lipoidosis of the myocardium might well be one result of feeding rapeseed oil rich in erucic acid, while other mechanisms might be involved in the occurrence of the focal histiocytic lesion. It is suggested by Ernster that the diminished mitochondrial respiration resulting from decreased β-oxidation of the fatty acids could lead to an accumulation of lipid droplets. This event could cause nutritional damage to the muscle fibres, with consequent necrosis. This muscle fibre necrosis, in turn, could provoke histiocytic reactions, occurrence of macrophages and proliferation of fibroblasts, and the final result would be an unspecific scar. A direct effect on the cell organelles as a result of accumulation of free fatty acids in the cytoplasm has also been proposed as the cause of cell death (16).

When discussing the small focal myocardial lesions observed in our control rats it might be of some interest to point out the observation by Schlesinger and Reiner (1955) that focal myolysis may be a predominant or incidental lesion in non-coronary diseases of man. The lesion has been ascribed to a variety of aetiological factors, suggesting that it constitutes the final morphological pathway for many different aetiological agents.

As mentioned previously we have no direct evidence of the effect of rapeseed oil in human pathology. However a large number of animals have been shown to react in the way we have described above, with fatty accumulation in the heart muscle cells, subsequent occurrence of histiocytic foci, and finally scarring of the myocardium. Such species include the common laboratory animals as well as monkeys, pigs and ducklings. It therefore seems reasonable to assume that the human heart will react in a similar manner. It is also of interest to mention that different animals have different susceptibilities. Furthermore, young animals are especially sensitive and male animals react more strongly than females.

It has been reported that the survival rate of animals fed high levels of rapeseed oil does not differ significantly from that of controls (17). It should be pointed out, however that in studies on rats fed large amounts of rapeseed oil over two years, 100 % of the animals exhibited myocardial fibrosis and a tendency to increased scarring with time (1). In this context the information presented by Beare Rogers (1972) on "stressed" rats is of interest. She found that among rats placed at an ambient temperature of +4 C for one to four weeks, the mortality was considerably higher for those given rapeseed oil than for the controls.

When considering all these experimental data it seems to us that rapeseed oil containing erucic acid must be regarded of potential relevance in human pathophysiology also. Furthermore, it has been claimed that erucic acid homologues and isomeres also provoke similar pathological effects in experimental animals (16). Such fatty acids are present, *inter alia*, in certain marine oils.

Acknowledgement

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Morphological Effects of Rapeseed Oil in Rats

III Studies in germ free rats

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Abstract

The morphological effects on the myocardium of feeding rapeseed oil were compared in conventional and germ-free rats in short term experiments (10 days). It was concluded that the fatty accumulation in the heart muscle cells occurring in rats fed rapeseed oil was not influenced by the presence or absence of a normal intestinal flora

In long-term experiments (80 days) under similar conditions, the myocardial effects of feeding germ-free rats with conventional rapeseed oil, rapeseed oil from the Canadian cultivar Oro very low in erucic acid, or arachis oil were studied in serial sections. Severe myocardial lesions developed in the group of rats fed conventional rapeseed oil, while in the other two groups the myocardium was completely normal These results give no support to the theory that other factors than C22.1 acids in rapeseed oil are responsible for the myocardial lesions.

Introduction

Feeding of rapeseed oil to rats and other experimental animals causes a series of pathological changes (1). These include alterations of the myocardium consisting of fatty accumulation in the muscle fibres, in a short time followed by myolysis and necrosis and later by fibrosis leading to multiple scars in the heart muscle. These effects have been referred to the high content (40—50 %) of erucic acid (C22.1) in conventional rapeseed oil. When a Canadian rapeseed oil, canbra oil, with an erucic acid content of only 2 % was fed to rats, however Rocquelin et al (7) observed myocarditis after eight weeks. Abdellatif and Vles (3 and 4), on the other hand, found only mild pathological changes in the myocardium after feeding Canbra oil to rats for 24 weeks. The changes were of the same nature and extent as those observed in a control group fed sunflower seed oil. Engfeldt and Brunius (6) made similar observations of small infiltrations of histiocytes after serial sectioning of hearts of rats fed arachis oil for 80 days. This gave a final concentration of 0 01 % of C22.1 acids in the diet. In other series of investigations by the same authors no lesions were seen in rats on diets containing canbra oil from the Canadian cultivar Oro or arachis oil after 80 or 160 days. It is conceivable that these differences in morphological changes in the myocardium between different laboratories might be due to other factors than the erucic acid content of the diet, such as latent infections, other dietary components or environmental conditions. It should be possible to approach the problem of latent infections by using germ-free animals. The aim of the present investigation was to study the effects of dietary fat with high and low contents of erucic acid on the myocardium of germ-free rats in short and long-term experiments.

Material and methods

Experimental design

In a short-term experiment (first series) conventional rapeseed oil was fed for 10 days to 5 germ-free and 5 conventional rats. A similar number of each type of rat was fed a control diet in which the fat source was

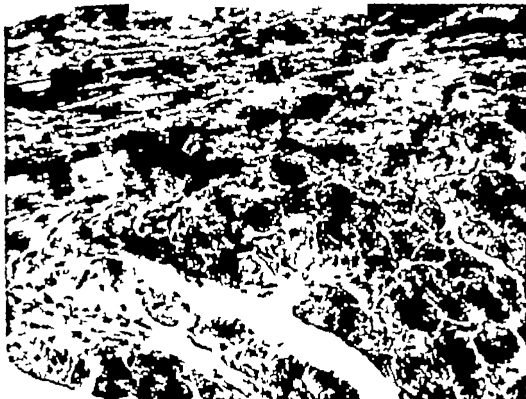


Fig 1 Photomicrograph of frozen section of ventricular wall of left heart from germ-free rat, stained with Scharlach Rot. The animal was fed rapeseed oil corresponding to 10.3 % w/w erucic acid [illegible]2–1) in the diet for 10 days. Numerous lipid droplets of varying [illegible] are seen in the muscle fibres. In certain areas the longitudinal [illegible] is easily discernible. × [illegible]70

arachis oil. In a long-term experiment (second series) conventional rapeseed oil with a high erucic acid content and rapeseed oil from the Canadian cultivar Oro with low erucic acid content were fed for 80 days to two groups of 6 germ-free rats each one group of 7 germ-free rats given arachis oil in the diet served as controls.

Animals

The germ-free rats were of Sprague Dawley origin and had been kept outbred for several generations. The con[illegible]entional animals were offspring of a colony of SPF rats (Anticimex, Ltd.) which originated from ex-germ-free animals of our germ-free strain. After being deli[illegible]ered, the con[illegible]entional animals were kept isolated without strict SPF conditions. The germ-free animals were maintained as described by Gustafsson (7). The first series of experiments was performed in a jacket isolator and the second in conventional isolators. Both germ-free and conventional rats were kept on raised bottom screens. Male rats 40 days old were used in the first series. The second series comprised both male and female rats, 35 days old. The animals were weighed once a week. Litter mates were as evenly distributed among the groups as possible.

Diets

In the first series the diets contained 40 cal [illegible] fat. They were composed of casein [illegible]2 g,

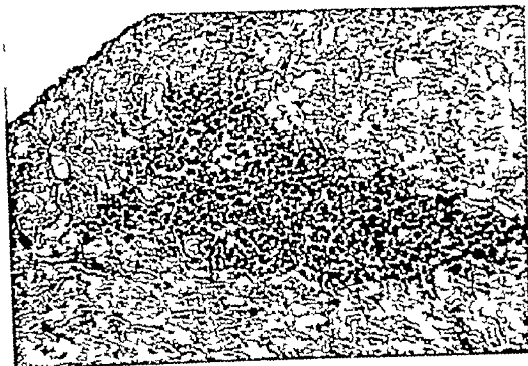


Fig. –. Photomicrograph of formalin-fixed paraffin-embedded section of ventricular wall of left heart from a germ-free rat. The animal was fed rapeseed oil corresponding to 4.0 % w/w erucic acid in the diet for 80 days. Disintegration of muscle fibres is evident and some fibres have been replaced by histiocytes, macrophages and lymphocytes. Staining according to Weigert Hansen. × 230.

vitamin mixtures[1] 2.5 g, minerals USP XVII (8) 5 g, cellulose flour 1 g, fat 21 g and wheat starch to make up to 100 g.

In the second series the diets contained 20 cal% fat, and the other components were included in the same amounts as in series 1. The fats contained in the different diets were analysed for fatty acids by gas chromatography (5). In the first series conventional rapeseed oil with 49.2 % C22:1 acids was used, and arachis oil containing 1.– % C22:1 acids was given to the control group. In the second series conventional rapeseed oil with 40.1 % C22:1 acids, rapeseed oil from the Canadian cultivar Oro with 0.3 % C22:1 acids and arachis oil with 0.1 % C22:1 acids were given to the three different groups.

The diets were mixed with 30 % water, granulated and sterilized in 2 cm layers by autoclaving with two vacuum cycles for 30 min at 121 C. The conventional animals were given their diet out of the same sterilized batch as the germ-free animals.

[1] The vitamin mixtures provided the following amounts of vitamins in mg per 100 g diet: Vitamin A palmitate 1.4, calciferol 0.01, dl-α-tocopherylacetate 50, phylloquinone 1, thiamine mononitrate 5, riboflavin 2, pyridoxine hydrochloride –, niacinamide 20, calcium pantothenate 10, p-aminobenzoic acid 30, biotin 0.1, pteroylglutamic acid 2, cyanocobalamin 0.002, choline chloride 200, inositol 100, ascorbic acid 100.

Morphological investigations

In the first series four frozen sections from each heart were stained in colloidal Scharlach Rot and haematoxylin. After paraffin embedding, sections were also stained with haematoxylin-eosin.

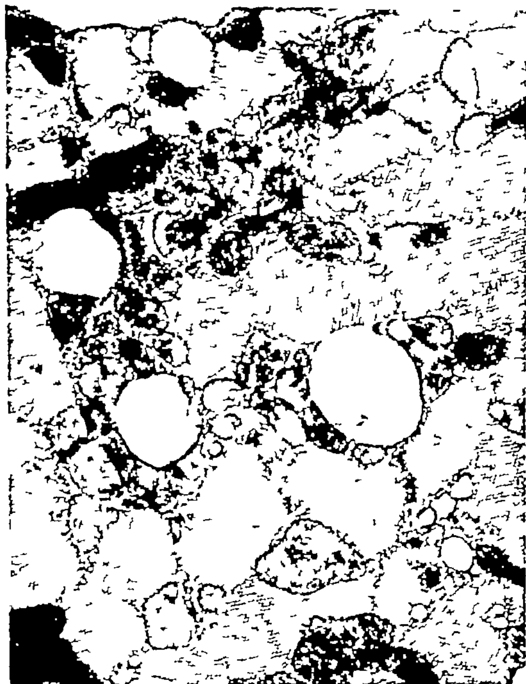


Fig. 3 Electron micrograph of ultrathin transverse section of left ventricular wall from germ-free rat fed rapeseed oil corresponding to 4.0 % w/w erucic acid in the diet for 80 days. An increased number of lipid droplets intermingled with the mitochondria is observed. These droplets are distorting the outline of the adjacent mitochondria. But apart from this finding the ultrastructure is normal. Staining with uranyl acetate and lead citrate × 15 000.

In the second series the hearts were serially sectioned after paraffin embedding. The sections were stained according to Weigert-Hansen. In one animal from each group the heart was perfused with _ % glutaraldehyde according to the technique used by Engfeldt and Brunius (6).

Results

Fat stained *frozen* sections of the hearts showed numerous lipid droplets in the 5 germ-free and 5 conventional animals after 10 days on a diet containing 10.3 % w/w erucic acid (C 22:1) (Fig 1). No fat droplets were seen in either the 5 germ-free or the 5 conventional rats on the diet containing arachis oil, *i.e.* 0.__ % w/w erucic acid. *In* the 80-day experiment the myocardium of the rats on rapeseed oil from the Canadian cultivar Oro and arachis oil appeared completely normal in the light and electron *microscopes. One of the males in the group* receiving conventional rapeseed oil (4.0 % C22.1 w/w in the diet) had extensive histiocytic infiltration with lesions scattered over the entire heart (Fig 2). In the other male and in one female, minor areas with histiocytic infiltration and fatty accumulation were seen *(Fig 3).* The hearts of the three remaining females showed no pathological changes.

Discussion

The extensive fatty accumulation in the *myocardial cells in* the rats fed the conventional rapeseed oil for 10 days and the absence of such accumulation in the rats fed arachis oil, irrespective of microbial status, confirm earlier findings of the effect of feeding conventional rapeseed oil to rats. The experiment also demonstrates that the *presence or absence of the normal intestinal* flora does not influence the fatty accumulation.

Low frequencies of mild changes of the myocardium, consisting of small foci of histiocytes and fibrosis, have been observed in some groups of animals but not in others *in long-term feeding experiments with rape* seed oil containing low contents of erucic acid or other oils with small amounts of C22.1 acids. This has caused difficulties in interpreting the effects of diets with erucic acid contents closer to the magnitude equivalent experienced in human consumption. In the present long-term studies no such lesions were found in germ-free rats fed a diet with rapeseed oil from the Canadian cultivar Oro as the fat source giving a final C22.1 acid concentration of 0.03 % in the *diet, nor in germ-free rats fed a diet with* arachis oil as the fat source, with a final concentration of C22.1 acids of 0.01 % in the diet. On the other hand, extensive lesions were found in the same series in one group *of germ-free* rats given conventional rapeseed oil with a concentration of 4.0 % C22.1 acid in the diet. Considering the fact that the negative findings rest on serial sections of the whole hearts of these animals, the present result provides strong evidence that the mild myocardial lesions sometimes found *in these oil feeding experiments could* be labelled "spontaneous" as postulated by Abdellatif and Vles (4). It is highly probable that these lesions might be related to microbial factors present in animals bred under conventional conditions. As the germ-free animals on the diet with 4.0 % C22:1 acids *from rapeseed oil showed extensive lesions,* when no lesions were found in the litter mates given 0.03 % C22.1 from rapeseed oil, the theory that other factors than the C22:1 acids in the rapeseed oil may be *responsible for the myocardial lesions* (2) is not corroborated by these studies.

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Electrocardiogram and Renal Concentrating Capacity in Rats Fed a Diet Containing Rapeseed Oil

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Abstract

The functional effects of a diet containing rapeseed oil (40 % of total energy intake) were studied in rats, starting at age 28 days. There were no effects on the electrocardiogram in spite of morphological changes in the myocardium. In 10 female rats the urine osmolality following 16 hours of dehydration was approximately 20 % lower than in 10 control rats during the 9th, 10th and 20th week of the experiment. It is suggested that erucic acid in the rapeseed oil inhibits β-oxidation of fatty acids in the kidney thereby depriving the kidney of energy involved in sodium transport

Introduction

In long-term experiments in rats rapeseed oil diets containing erucic acid (C 22 1 Δ 13) produce myocardial lesions initially as intracellular lipidosis, later histiocyte infiltration and finally fibrosis. Rapeseed oil diets also produce renal effects in the rat, as shown after 16 weeks by increased kidney weight, tubular dilatation and focal connective tissue degeneration (1). In an effort to find functional tests which would enable us to monitor the effects of erucic acid continuously in living animals the electrocardiogram (ECG) and the renal concentrating capacity were investigated in rats fed a diet containing rapeseed oil.

Methods

The experimental group consisted of 10 male and 10 female rats. They were fed a diet containing rapeseed oil equivalent to 40 % of the energy content (10 % erucic acid w/w). The control group consisted of the same number of animals. Their diet contained arachis oil equivalent to 40 % of the energy (0.4 % erucic acid w w) The animals were 28 days old at the start of the experiment All animals were housed in individual cages

The ECG was recorded on all male rats on the 4th day of the experiment and on all female rats on the 5th day From then on the ECG was recorded on every rat once a week for 8 weeks. Standard leads I, II and III were recorded via finegauge cannulae inserted hypodermically in the extremities while the rat was kept reasonably immobilized in a narrow cage. The P R and QRS intervals and heart rate were measured, and the results were analyzed statistically The electrocardiograph was a Mingograph-34 (Siemens-Elema, Solna, Sweden)

Because of difficulties in emptying the urinary bladder of the male rats, these were excluded from the experiment. The renal concentrating capacity was thus investigated on female rats only on weeks 9 10 and 20 of the experiment. Water was withheld from the rats over night (from 4 PM). Urine samples were obtained at 8 AM the following morning by compression over the urinary bladder The samples were immediately cooled, and the freezing point was repeatedly measured on a volume of 0 1 ml on a Knauer osmometer type M.

Results

There were no definite differences between the ECG s of the test groups (Table 1)

TABLE 1

ECG data. 10 rats in each group (mean ± standard deviation)

Group	Frequency beats/min	P—R msec	QRS msec
Male rats			
Control week 1	486 ± 44	4 ± [illegible]	14 ± 3.0
Erucic acid week 1	441 ± 4	47 ± [illegible]	13 ± 1.[illegible]
Control week 9	49 ± 55	46 ± 3.3	16 ± 4.6
Erucic acid week 9	480 ± 95	44 ± [illegible]	12 ± 4.4
Female rats			
Control week 1	504 ± 42	41 ± 2.3	13 ± 3.5
Erucic acid week 1	492 ± 47	43 ± 2.1	12 ± 3.0
Control week 9	516 ± 64	4 ± 3.0	12 ± 3.0
Erucic acid week 9	516 ± 51	43 ± [illegible]1	13 ± 3.7

TABLE [illegible]

Urine osmolality in female rats after 16 hours of dehydration (mean ± standard deviation)

	Week 9 mOsm/kg H_2O	10	20	[illegible]3—[illegible]5
Control diet	[illegible]6 ± 196 (n = 6)	6?0 ± 176 (n = 6)	620 ± 480 (n = 8)	134 ± ?08 (n = 7)
Erucic acid	17 3 ± 268 (n = 7)	109 ± 145 (n = 6)	?098 ± 297 (n = 6)	
Erucic acid 0 week + control diet 5 weeks				?031 ± 165 (n = 7)
Student's t-test	$p < 0.005$	$p < 0.001$	$p < 0.0[illegible]5$	N.S.

The renal concentrating capacity was significantly lower in the rapeseed oil group in comparison with the control group (Table).

Five females of the rapeseed oil group were transferred to control diet after week [illegible]0. Tests on renal concentrating capacity during weeks [illegible]3—[illegible]5 did not any longer reveal any difference from the original control animals. Whether the slightly diminished concentrating capacity the original control animals during this period was due simply to change of personnel (which did occur at the time) or to other environmental factors was not resolved.

The animals were killed after completion of the functional studies. There were no significant differences in weight of whole body or viscera between erucic acid and control animals. The weight of the heart in females exposed to erucic acid did however decrease significantly ($p < 0.0[illegible]5$) after the rats had been transferred to a control diet for 5 weeks.

The microscopic examination of the male rats after 10 weeks and of the female rats after 0 weeks revealed fatty infiltration in the heart in the erucic acid group. The five erucic acid rats that had been transferred to the control diet 5 weeks before killing did not have any fatty infiltration of the heart.

Four of the ?0 male rats had nephrocalcinosis when killed after 10 weeks, and all female rats had nephrocalcinosis

TABLE 3

Weight of organ of male rats, 10 in each group (mean ± standard deviation)

Group	Body wt g	Heart g	Liver g	Kidneys g	Testes g
Control diet 13 weeks	388 ± 16	1.15 ± 0.09	10.2 ± 0.70	2.4 ± 0.19	3.1 ± 0.34
Erucic acid 13 weeks	379 ± 22	1.20 ± 0.11	10.6 ± 0.89	2.8 ± 0.30	3.1 ± 0.25

TABLE 4

Weight of organs of female rats, 5 in each group (mean ± standard deviation)

Group	Body wt g	Heart g	Liver g	Kidneys g
Control diet 20 weeks	288 ± 24	0.77 ± 0.072	7.6 ± 0.57	1.6 ± 0.12
Erucic acid 20 weeks	278 ± 29	0.83 ± 0.052	8.0 ± 0.63	1.7 ± 0.18
Control diet 20+5 weeks	297 ± 29	0.78 ± 0.037	7.6 ± 0.61	1.7 ± 0.14
Erucic acid 20 weeks + control diet 5 weeks	*278 ± 32*	*0.74 ± 0.045* ($p < 0.025$ when compared to erucic acid 20 W)	*7.3 ± 0.80*	*1.6 ± 0.14*

Discussion

In spite of the marked morphological effects of rapeseed oil on the myocardium, there have been no reports on changes of the ECG. Another series with no effects on the ECG has been reported to us (Egg Larsen, N. personal communication). Evidently the conduction system of the hearts is not affected. On the other hand it has been shown that the contractile force of the isolated heart of the rat is decreased by feeding the rats a rapeseed oil diet (Egg Larsen, personal communication).

The renal concentrating capacity was slightly lowered in the female rats fed the rapeseed oil diet. The rats were tested only on weeks 9, 10 and 20 of the experiment. It cannot be excluded that a larger effect would be seen at an earlier stage, e.g. after one week when the fat infiltration in the myocardium is maximal. Later there might be some metabolic adaptation to the erucic acid.

The decreased concentrating capacity is most likely an effect of increased osmotic load in the urine due to inhibition of the energy-requiring reabsorption of sodium, rather than a specific effect on the concentrating mechanism. *In vitro* studies suggest that a considerable fraction of renal O_2 consumption represents the oxidation of longchain fatty acids (2). It is suggested that erucic acid may *increase urinary excretion* of electrolytes by inhibiting β-oxidation of fatty acids in the kidney.

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Observations on Lipid Composition with Particular Reference to Cardiolipin of Rat Heart after Feeding Rapeseed Oil*

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Abstract

The influence of dietary rapeseed oil on the lipid classes and fatty acid pattern of rat heart homogenate and mitochondria has been investigated after feeding a diet with 9.8 weight % erucic acid for 10 days and 1.4 and 2.6 % erucic acid for 28 days.

The rats treated with 9.8 % erucic acid showed a significant increase in the triglycerides of the heart mitochondria. This tendency was much less pronounced in rats treated with 1.4 resp. 2.6 % erucic acid. These results confirm those of other investigators. A slight increase in the cholesterol esters of the mitochondria could be seen in all the treated rats.

The total phospholipids were decreased in the experiment with 9.8 % erucic acid and slightly increased in experiments with 1.4 and 2.6 % erucic acid. The concentration of phosphatidylcholine showed a tendency to increase and the concentration of phosphatidylethanolamine to decrease in the experiment with 9.8 % erucic acid in the diet. The concentration of cardiolipin was mainly unchanged.

In all experiments the triglycerides of the heart mitochondria showed a high content of erucic acid. The fatty acids of the cholesterol esters of the heart mitochondria were also influenced of dietary rapeseed oil but to a less extent than the triglycerides. The fatty acids of phosphatidylcholine, phosphatidylethanolamine and cardiolipin were all influenced by the dietary rapeseed oil, but the erucic acid seemed to have a specific affinity to cardiolipin.

Cardiolipin of rat heart mitochondria was isolated and identified with gas chromatography and mass spectrometry. The isolated cardiolipin was found to contain 12 per cent erucic acid after feeding 9.8 % erucic acid as rapeseed oil for 10 days. Similar results were obtained after feeding glyceryl trierucate for 5 days to rats. The incorporation of erucic acid into cardiolipin was followed by a corresponding decrease of linoleic acid. This observation is of great interest because the molecular structure of fatty acids in lipid molecules has a profound influence of the packing of these molecules in a bilayer. Since cardiolipin is a component of the inner membrane of mitochondria its high affinity for erucic acid might influence the normal function of the inner membrane of heart mitochondria.

Introduction

Roine et al. (25) were the first to show that rats fed rapeseed oil for two to three months showed foci of histiocyte infiltration in the myocardium. Abdellatif and Vles (1) investigated the pathological effects of dietary rapeseed oil in rats. Fatty acid infiltration was found in heart, skeletal muscle and adrenals after feeding 60 Cal % of rapeseed oil for only two weeks, Houtsmuller *et al.*

* Part of this investigation has been published in Lipids, 9 771—780, 1974.

(19) investigated the amount and composition of the lipid classes in the heart of rats fed a diet containing 50 Cal % rapeseed oil for periods varying from 1 day to 6 weeks. A sharp increase in lipid content was observed after 3 days on the diet, which is mainly due to an increase in triglycerides. An increase in the content of free fatty acids was also observed. Of the total lipids 27 % of the fatty acids was erucic acid. Studies of heart mitochondria in vitro revealed that the rate of ATP synthesis is lower after feeding an erucic acid containing diet than for a diet containing sunflower oil. The degree of inhibition was roughly proportional to the erucic acid content of the diet.

It has been suggested by several authors (17 ... 6, 30) that the phospholipids play a major role in the maintenance of normal function within the cell membrane. The reason for the occurence of mixtures of different phospholipid classes and the variation in the fatty acid composition from one type of membrane to another is not yet understood.

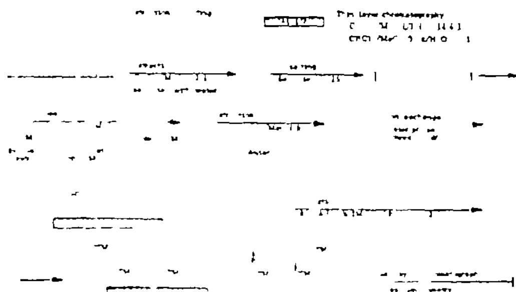
Considerable study has been given to the role of unsaturated fatty acids in mitochondrial function (13). There is evidence that changes in the fatty acid composition are fundamental to metabolic and physical differences in mitochondria. Recently a close correlation between the alteration of cardiolipin and mitochondrial ATP ase activity has been reported, indicating the excistence of specific association between this enzyme and cardiolipin, independently of other phospholipids (27).

Cardiolipin is a characteristic phospholipid of heart mitochondria and cardiolipin normally contains a high concentration of linoleic acid. We have investigated the phospholipid composition and fatty acid pattern of rat heart mitochondria in a series of experiments where the erucic acid content in the diet and the feeding time were varied as described by Engfeldt and Brunius (7).

The results indicate that erucic acid is incorporated into several phospholipids of the rat heart but that erucic acid seems to have a specific affinity to be incorporated into the cardiolipin molecule of the rat heart mitochondria.

Material and methods

Five groups of 10 male and 10 female four weeks old Sprague-Dawley rats in each group were fed a diet containing 40 Cal %



of fat. The feeding time and the erucic acid content were varied, one group was fed 9.8 % erucic acid for 10 days and two groups 1.4 % and 2.6 % erucic acid for 28 days. The erucic acid was given as rapeseed oil. The remaining two groups were fed peanut oil instead of rapeseed oil. Further information of the composition of the diets is given by Engfeldt and Brunius (7). Further two groups of rats have been investigated. One group of 10 rats fed a basic diet consisting of pellets with an addition of 1.85 g trierucate (AB Karlshamns Oljefabriker Karlshamn, Sweden) per day given by tube for 5 days. The other group was fed only basic diet.

The purity of glyceryl trierucate (AB Karlshamns Oljefabriker Karlshamn, Sweden) used in our experiments was investigated by gas liquid chromatography (GLC). The total fatty acids of trierucate was found to contain 92.5 % erucic acid (C 22:1), 2.5 % gadoleic acid (C 20:1) and 5 % of other fatty acids.

Preparation of homogenate and isolation of mitochondria

Rat heart homogenate was obtained by homogenization in ice-cold 0.25 M sucrose containing 1 mM neutralized EDTA. Rat heart mitochondria were isolated by ultracentrifugation (16). Mitochondria and homogenate were extracted according to Folch (11). Protein content was analysed with micro Kjeldahl (9).

Separation of total lipids into different lipid classes using silicic acid chromatagraphy

Separation on silicic acid was carried out mainly as described previously (4). About 10 mg of total lipids dissolved in n-hexane were placed at the column. Three fractions were eluted using the following elution mixtures.

1	Pentane/Benzene 85:15	(Cholesterol esters are eluted)
2.	Chloroform	(Glycerides, cholesterol and free fatty acids are eluted)
3	Methanol	(Phospholipids and other polar lipids are *eluted*)

Separation of phospholipids by thin layer chromatography (TLC)

Preparation of adsorbent

The plates were prepared with an automatic TLC-coater (Camag, Muttenz, Switzerland). Air-dried Silica Gel H (Merck, Darmstadt, Germany) plates (20 × 20 cm and 0.25 mm layer thickness) were activated for 2 hours at 110 C before use.

Sample application

About 7 mg of extracted lipids dissolved in 100 μl chloroform/methanol 2:1 were applied over a 5 cm line with a 100 μl Hamilton syringe.

Solvent systems

1 Chloroform/methanol/ 25 % aqueous ammonia 14 6.1

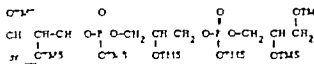
2. Chloroform/methanol/acetic acid/water 80·13.8·0.3

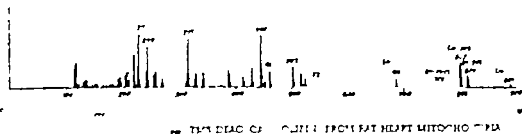
Development of chromatograms

200 ml of the desired solvent mixture was placed in the chromatography chamber which was lined on the sides with filter papers. The paper liners were saturated for about 0.5 h before insertion of the plates. The plates were first developed with solvent system 1 to a height of 12 cm from the application line. After air drying for 0.5 h the plates were developed with solvent system 2 to a height of 17 cm.

Detection

The bands were partly scraped from the plates. The ends of each side of the 5 cm line were saved for detection. The detection reagent was $(NH_4)_2SO$ 100 g and H_2SO 5 ml made up to a volume of 500 ml with water The plates were charred for 90 min





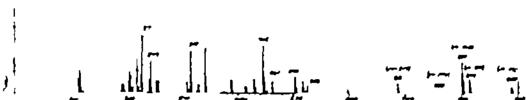


Fig. _a. Mass spectrum of TMS Deac-Cardiolipin (standard).
b. Mass spectrum of TMS Deac-Cardiolipin of rat heart mitochondria from rats fed a diet containing _6 % erucic acid for 3 days.

at 180 C. The R_f -lues of the separated b nds were compared with R_f alues of purchased phospho p

Densitometry

The relati e contribu n of ma among TLC separated phosp pid w est ma ed by measuring the light bsorption the charred phospho pid band m V a n TLD 100 (Vitatron, Dieren, H

Fatty acid analysis

The relati e distr bution of f tty acids in cholesterol esters, triglycerides and phosphatidylethanolamine phosphatidylcholine a d c rdiol pin from the TLC-separated phospholipids has been invest gated. The l d were hy rolysed in acid methanol and th f tty acids were con erted to their f tty acid met lesters with 2,2-dimethoxypropan (1). The f tty acid methylesters were se p ed nd ident ied by g liquid chro-

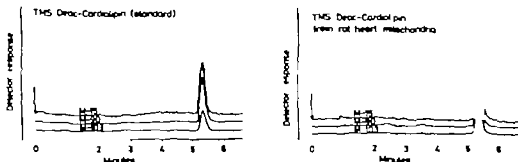


Fig 3 a. Mass fragmentogram of TMS Deac-Cardiolipin (standard).
b. Mass fragmentogram of TMS Deac-Cardiolipin of rat heart mitochondria from rats fed a diet containing 9.8 % erucic acid for 10 days.

matography using a 4 m × 4 mm ID glass column packed with 3.5 % EGSS-X on acid-washed and silconized Chromosorb W (100—120 mesh). The different peak areas were calculated, and the results were expressed as area percentages. The identity of erucic acid incorporated in different lipids was confirmed by GC MS using purchased erucic acid as reference.

Isolation and identification of cardiolipin

Cardiolipin was separated from the other phospholipids by thin layer chromatography as described above. The following isolation and identification procedures are summarized in Fig. 1

Sephadex column chromatography

The supposed cardiolipin band was eluted from the adsorbent by shaking vigorously with 2 × 10 ml of chloroform/methanol 2:1 saturated with water followed by filtration (sintered glass filter medium porosity). The filtrate was evaporated in the cold by means of a rotary evaporator. The lipid may be contaminated with adsorbents and salts. These contaminations were removed by passing the sample through a 10 cm × 1 cm ID Sephadex column according to Fleischer and Rouser (10).

Elution mixtures:

1. Chloroform/methanol 19:1 saturated with water
2. Methanol/water 1:1

Solvents for 1 are mixed several times in a separatory funnel. The clear lower phase is used.

Preparation of column.

A slurry of Sephadex G 25 in methanol/water 1:1 was equilibrated over night and degassed before use. The column 1 cm ID was packed to a height of 10 cm. The column was then washed through two cycles with 50 ml of elution mixture 1 and 2 and then with further 50 ml of elution mixture 1

Sample application.

The lipid was dissolved in a small volume of chloroform/methanol 19:1 saturated with water before transferred to the column.

Elution of column.

The sample was eluted with 50 ml of elution mixture 1 followed by 50 ml of elution mixture 2 in order to clear the column from salts.

Deacylation

The solvent was evaporated in the cold by means of a rotary evaporator. The lipid was

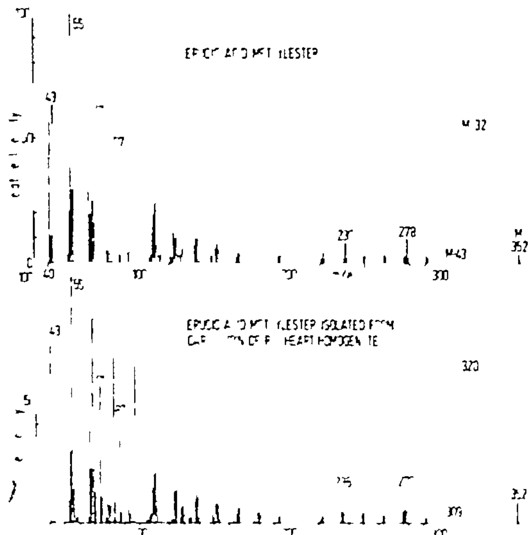


Fig 4 a M ss pectrum of erucic acid methylester (ta dard)
b Ma spectrum of erucic acid methylester isolated from cardi pin of rat heart homogenate

weighed nd d sol ed in 1 ml of chloroform methanol 1 4 ??) µl 1 . M NaOH in methanol water 1 1 was added to gi e a final concentr on of 0 M The mixture was incub ed for ten m utes at 37 C The re tion w s term ed by first coo ng in ice and then neut z g with 1 M acetic acid (?60 µl ar req red   ml of chloroform methanol 9 1   ml of w ter nd 1 ml of nobutanol were add d The m ture w   shaked pround   d centr f ged for 20 m utes at 2500 rpm The w er phase con taining the deacyla d l p d w   con erted to the free acid form on Dowex 50 W X 8 column which w   newly loaded w th 1 M N OH and 1 M acetic acid (6)

Gas chromatography

Silylation

The sol ent wa es por ted t room tem perature by mean of a rotary evapor ter nd dried in acuum over P O The dried deacyla ed lipid w   con erted to it trime thylsilyl deri t with N O bis (t imethyl silyl) tr fluoroacetamide (BSTFA) + 10 % trimethylchlorosilane (TMCS) dry pyridine

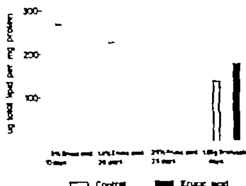


Fig 5 Total lipid content in rat heart *mitochondria* from rats fed a diet of 40 Cal % fat containing 9.8, 1 4 and 2.6 % erucic acid given as rapeseed oil for 10, 28 resp. 28 days and in *homogenate* of rat heart from rats fed a diet consisting of pellets with an addition of 1.85 g trierucate per day for 5 days.

..l About 300 µl was added and the mixture was allowed to react for 1 hour at 70 C. If the reaction failed further 20 µl of TMCS were added and the mixture was allowed to stand for further 1 hour at 70 C. About 12 µl of the reaction mixture were injected to the GLC-column.

Preparation of GLC-column.

The GLC-column was prepared according to Horning (8). The column used was a 0.5 m × 4 mm ID glass column packed with 1 % Se 30 on acid-washed and silliconized Chromosorb W (80—100 mesh) and filled at the top with 10 % Se-30 to a height of 2 cm

Mass spectrometry

The mass spectrometer used was a LKB 9000 gas chromatograph-mass spectrometer (GC MS), (LKB Produkter Stockholm—Bromma, Sweden)

The trimethylsilyl deacylated cardiolipin (TMS deac-cardiolipin) was eluted after about 6.5 minutes when using temperature programme 180— 40° C, 4 C per minute. Flow rate about 20 ml per minute.

Spectra were obtained at 70 eV ionizing potential, a trap current of 60 µA, accelerating voltage 3.5 kV ion source 250° C, molecule separator .30 C, flash heater .50 C, scan speed 6, UV-paper speed 100 mm per sec., filter 120 cps, electron multiplier sensitivity 125 and slits 0.2 and 0.3 mm.

The unknown spectrum was normalized and compared with a spectrum of reference cardiolipin treated in the same way as the unknown.

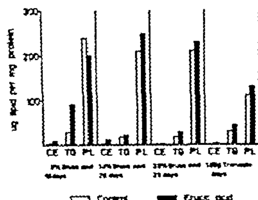
Mass fragmentography

When only small amounts of sample were available cardiolipin was identified by mass fragmentography Three fragments were focused on the multiple ion detector (MID) namely those having the highest mass numbers: m/e=889 (M 15), m/e=814 (M-90) and m/e=801 (M 103). They have been detected on channel 1 2 resp 3 with an amplification of 900 × 300 × resp 90 × respectively Filter was 0.25 cps on each channel, electron multiplier sensitivity 110 and measuring time 20 msec. All other data were the same as when taking mass spectra except electron energy which was only 20 eV when using the MID The three ions were detected simultaneously and had equal retention time.

Results

Identification of cardiolipin

Cardiolipin of heart mitochondria from rats fed a diet containing 1 4 and 2.6 % erucic acid for 28 days was identified by GC-MS.



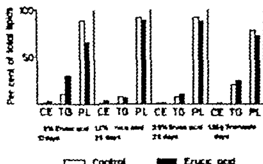


Fig 6 The content of cholesterolesters (CE), triglycerides (TG) and phospholipids (PL) in rat heart *mitochondria* from rats fed a diet of 40 Cal % fat containing 9.8, 1.4 and 2.6 % erucic acid given as rapeseed oil for 10, 28 resp. 28 days and in *homogenat* of rat heart from rats fed a diet consisting of pellets with an addition of 1.85 g trierucate per day for 5 days.

Fig 7 Relative distribution of the different lipid classes cholesterolesters (CE), triglycerides (TG) and phospholipids (PL) in rat heart *mitochondria* from rats fed a diet of 40 Cal % fat containing 9.8, 1.4 and 2.6 % erucic acid given as rapeseed oil for 10, 28 resp 28 days and in heart *homogenate* from rats fed a diet consisting of pellets with an addition of 1.85 g trierucate per day for 5 days.

Only one peak was obtained at the gas chromatogram. A reasonably good agreement could be obtained when comparing the spectrum of TMS deac-cardiolipin from rat heart mitochondria with the spectrum of a standard (Fig. 2). The two spectra differ however slightly in base peak. The fact that cardiolipin has three asymmetrical carbon atoms which give rise to different optical isomers may be the cause of the different fragmentation. An other explanation is that there can be a small amount of contaminations in the purified sample despite the rigorous isolation and purification procedures described in Fig. 1. In that case the intense fragment at m/e = 147 partly comes from the contammation.

Cardiolipin of heart mitochondria from rats fed a diet containing 9.8 % erucic acid for 10 days was identified by mass fragmentography. Mass fragmentograms of isolated TMS deac-cardiolipin and standard are compared in Fig. 3. Sample and standard have equal retention time and the ratios of the peak heights are the same.

Identification of erucic acid

The identity of erucic acid isolated from cardiolipin of rat heart homogenate was confirmed by GC-MS. The mass spectra of erucic acid from cardiolipin and purchased erucic acid are compared in Fig. 4.

Total lipid content in homogenate and mitochondria of rat heart

The influence of rapeseed oil diet on the total lipid content of homogenate and mitochondria of rat heart is given in Table I and Fig. 5. An increase in the total lipid content per mg protein can be seen after both short term and long-term feeding.

The distribution of different lipid classes in homogenate and mitochondria of rat heart

The distribution of cholesterol esters, triglycerides and phospholipids in homogenate and mitochondria of rat heart are shown in

TABLE I.

Total lipid content in rat heart *mitochondria* f om rats fed a diet of 40 Cal % fat containing 9.8, 1.4 and 2.6 % erucic acid given as rapeseed oil for 10 28 resp. 28 days and in *homogenate* of rat heart from rats fed a diet consisting of pellets with an addition of 1.85 g trierucate perday for 5 days.

	Erucic acid in diet	Days	µg lipid/mg protein	% increase
Control	0.2 %	10	270	12
Exp	9.8 %	10	300	
Control	0.2 %	28	230	24
Exp	1.4 %	28	280	
Control	0.2 %	28	230	14
Exp	2.6 %	28	260	
Control	—	5	140	22
Exp	1.85 g trierucate per day	5	180	

TABLE II.

The content of cholesterolesters (CE), triglycerides (TG) and phospholipids (PL) in rat heart *mitochondria* f om rats fed a diet of 40 Cal % fat containing 9.8 1.4 and 2.6 % erucic acid given as rapeseed oil for 10 28 resp. 28 days and in *homogenate* of rat heart from rats fed diet consisting of pellets with an addition of 1.85 g trierucate per day for 5 days.

	Erucic acid in diet	Days	µg lipid/mg protein CE	TG	PL
Control	0.2 %	10	1	28	240
Exp	9.8 %	10	8	92	200
Control	0.2 %	28	1	17	210
Exp	1.4 %	28	11	21	250
Control	0.2 %	28	1	17	210
Exp	2.6 %	28	2	29	230
Control	—	5	traces	30	110
Exp	1.85 g trierucate per day	5	1	44	130

TABLE III.

Relative distribution of the different lipid classes cholesterol esters (CE), triglycerides (TG) and phospholipids (PL) in rat heart *mitochondria* from rats fed a diet of 40 Cal % fat containing 9.8 1.4 and 2.6 % erucic acid given as rapeseed oil for 10, 28 resp. 28 days and in rat heart *homogenate* f om rats fed a diet consisting of pellets with an addition of 1.85 g trierucate per day for 5 days.

	Erucic acid in diet	Days	% CE	% TG	% PL
Control	0.2 %	10	0.5	10.6	88.9
Exp	9.8 %	10	2.8	30.7	66.5
Control	0.2 %	28	0.5	7.6	91.9
Exp	1.4 %	28	3.9	7.3	88.8
Control	0.2 %	28	0.5	7.6	91.9
Exp	2.6 %	28	0.7	11.2	88.1
Control	—	5	0.1	20.7	79.2
Exp	1.85 g trierucate per day	5	1.3	25.2	73.5

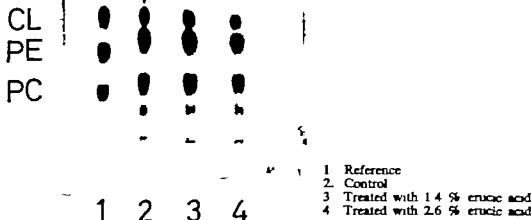


Fig 8. Thin layer chromatogram showing separation of phospholipids into phosphatidylcholine (PC), phosphatidylethanolamine (PE) and cardiolipin (CL) of rat heart *mitochondria* from rats fed diet of 40 Cal % fat containing 1 4 and 2.6 % erucic acid given as rapeseed oil for 28 days.

Table II and Fig. 6. Rats treated with 9.8 % acid in the diet for 10 days show a ificant increase in triglycerides, while the phospholipids have a tendency to decrease. This accumulation of triglycerides in rat heart mitochondria after short-term feeding of rapeseed oil confirmes the results of other investigators (19 24). The tendency is much less pronounced in rats treated with 1 4 and ..6 % *erucic acid for* ..8 *days. The* total phospholipids slightly increase in this experiment.

The relative distribution of different lipid *classes is shown in Table III and Fig. 7* There is a tendency to a higher percentage of triglycerides in treated rats. The cholesterol esters show a less pronounced tendency to increase

The distribution of different phospholipids in homogenate and mitochondria of rat heart

A typical thin layer chromatogram of separated phospholipids is shown in Fig. 8. The three major phospholipid classes phosphatidylcholine, phosphatidylethanolamine and cardiolipin are well separated from each other The distribution of different phospholipids is shown in Table IV and Fig. 9 As shown in Tables IV—VI and Figs. 9—11 there is a tendency towards an increased concentration of phosphatidylcholine and a decreased concentration of phosphatidyl-*ethanolamine in mitochondria and homo-*genate of rat heart after feeding 9.8 % erucic acid as rapeseed oil for 10 days. The concentration of cardiolipin is mainly un-*changed. There is no significant difference* in the relative distribution of different phospholipids between mitochondria and homogenate of rat heart.

Composition of fatty acids of different lipid classes in homogenate and mitochondria of rat heart

The influence of rapeseed oil and trierucate on the fatty acid pattern in cholesterol esters,

TABLE IV

The content of phosphatidylcholine (PC), phosphatidylethanolamine (PE) and cardiolipin (CL) in rat heart *mitochondria* from rats fed a diet of 40 Cal % fat containing 9.8, 1.4 and 2.6 % erucic acid given as rapeseed oil for 10 28 exp 28 days and in *homogenate* of rat heart from rats fed a diet consisting of pellets with an addition of 1.85 g trierucate per day for 5 days.

	Erucic acid in diet	Days	µg phospholipids/mg protein PC	PE	CL
Control	0.2 %	10	87	109	42
Exp	9.8 %	10	137	30	32
Control	0.2 %	28	76	96	38
Exp	1.4 %	28	99	110	42
Control	0.2 %	28	76	96	38
Exp	2.6 %	28	86	110	33
Control	—	5	51	51	10
Exp	1.85 g trierucate per day	5	59	58	13

TABLE V

Relative distribution of phosphatidylcholine (PC), phosphatidylethanolamine (PE) and cardiolipin (CL) in rat heart *homogenate* from rats fed a diet of 40 Cal % fat containing 9.8 1.4 and 2.6 % erucic acid given as rapeseed oil for 10, 28 resp. 28 days and from rats fed a diet consisting of pellets with an addition of 1.85 g trierucate per day for 5 days.

	Erucic acid in diet	Days	% PC	% PE	% CL
Control	0.2 %	10	40.4	42.5	17.1
Exp	9.8 %	10	67.7	17.4	14.9
Control	0.2 %	28	38.7	48.1	13.1
Exp	1.4 %	28	44.5	40.8	14.7
Control	0.2 %	28	38.7	48.1	13.1
Exp	2.6 %	28	45.2	42.5	12.3
Control	—	5	45.0	45.2	9.8
Exp	1.85 g trierucate per day	5	45.5	46.4	8.5

TABLE VI.

Relative distribution of phosphatidylcholine (PC), phosphatidylethanolamine (PE) and cardiolipin (CL) in rat heart *mitochondria* from rats fed a diet of 40 Cal % fat containing 9.8, 1.4 and 2.6 % erucic acid given as rapeseed oil for 10 28 exp. 28 days.

	Erucic acid in diet	Days	% PC	% PE	% CL
Control	0.2 %	10	36.6	45.6	17.8
Exp	9.8 %	10	63.9	15.1	16.0
Control	0.2 %	28	36.4	45.7	17.9
Exp	1.4 %	28	39.3	44.0	16.7
Control	0.2 %	28	36.4	45.7	17.9
Exp	2.6 %	28	37.7	48.1	14.2

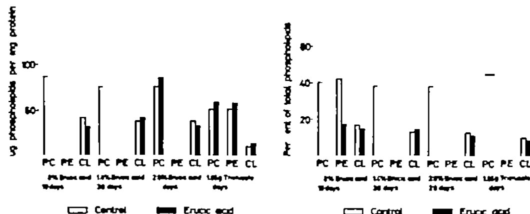


Fig 9 The content of phosphatidylcholine (PC), phosphatidylethanolamine (PE) and cardiolipin (CL) in rat heart *mitochondria* from rats fed a diet of 40 Cal % fat containing 9.8, 1 4 and 2.6 % erucic acid given as rapeseed oil for 10, 28 resp. 28 days and in *homogenate* of rat heart from rats fed a diet consisting of pellets with an addition of 1 85 g trierucate per day for 5 days.

Fig 10. Relative distribution of phosphatidylcholine (PC), phosphatidylethanolamine (PE) and cardiolipin (CL) in rat heart *homogenate* from rats fed a diet of 40 Cal % fat containing 9.8, 1 4 and 2.6 % erucic acid given as rapeseed oil for 10 28 resp. 28 days and from rats fed a diet consisting of pellets with an addition of 1 85 g trierucate per day for 5 days.

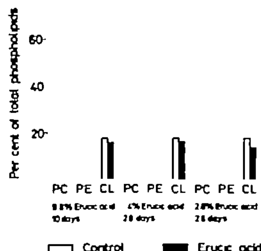


Fig 11 Relative distribution of phosphatidylcholine (PC), phosphatidylethanolamine (PE) and cardiolipin (CL) in rat heart *mitochondria* from rats fed a diet of 40 Cal % fat containing 9.8 1 4 and 2.6 % erucic acid given as rapeseed oil for 10 28 resp. 28 days.

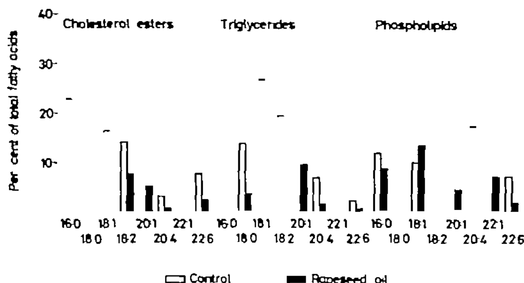


Fig. 12. Relative distribution of total fatty acids of different lipid classes in rat heart *mitochondria* from rats fed a diet of 40 Cal % fat containing 9.8 % erucic acid given as rapeseed oil for 10 days.

triglycerides and phospholipids is shown in Tables VII—IX and Figs. 12—14. Erucic acid is predominantly incorporated in the heart triglycerides. In the experiment with 9.8 % erucic acid the triglycerides of rat heart mitochondria contain 38 % erucic acid. In the experiments with 1.4 % and 2.6 % erucic acid the erucic acid content in the triglycerides are 3.2 and 7.0 % respectively. There is also an incorporation of erucic acid in cholesterol esters and phospholipids, but to a less extent than in triglycerides.

In the experiment with 9.8 % erucic acid the erucic acid content in the cholesterol esters is 26 % and there is a corresponding decrease in palmitic acid, oleic acid and linoleic acid content.

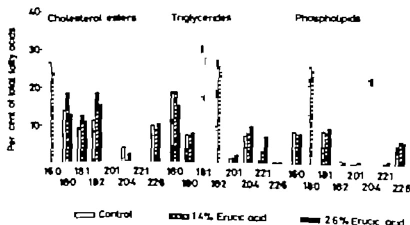


Fig. 13. Relative distribution of total fatty acids of different lipid classes in rat heart *mitochondria* from rats fed a diet of 40 Cal % fat containing 1.4 and 2.6 % erucic acid given as rapeseed oil for 28 days.

TABLE IX.

Relative composition of total fatty acids of different lipid classes in rat heart *homogenate* from rats fed a diet containing 1.85 g trierucate per day (given by tube) for 5 days.

Fatty acid	Cholesterol esters control	Cholesterol esters treated erucic acid 1.85 g	Triglycerides control	Triglycerides treated erucic acid 1.85 g	Phospholipids control	Phospholipids treated erucic acid 1.85 g
14:0	[illegible]5	2.6	1.7	1.8	0.1	0.1
16.0	18.9	15.8	23.7	18.8	12.1	8.3
16.1	9.3	6.3	4.3	3.6	0.7	0.6
17:0	1.5	3.7	0.9	0.8	0.7	0.4
18:0	7.6	4.8	9.8	7.4	[illegible]3.6	23.7
18.1	12.2	13.3	29.1	26.5	8.9	9.5
18.2	6.6	21.9	20.1	16.6	20.9	20.9
18.3	—	0.[illegible]	1.2	1.0	0.2	0.1
20.0	0.2	0.6	—	—	—	—
20:1	0.1	0.6	0.6	2.0	0.3	0.7
20.2	0.4	0.4	—	0.1	0.2	0.2
20.4	17.5	16.1	3.5	2.8	16.1	17.2
22.1	—	10.5	—	15.4	—	2.5
22:6	3.2	3.[illegible]	5.1	3.2	16.2	15.8

TABLE X.

Relative composition of total fatty acids of separated phospholipids in rat heart *homogenate* from rats fed a diet containing 9.8 % erucic acid for 10 days.

Fatty acid	Phosphatidylcholine control erucic acid 0.[illegible] %	Phosphatidylcholine treated erucic acid 9.8 %	Phosphatidylethanolamine control erucic acid 0.2 %	Phosphatidylethanolamine treated erucic acid 9.8 %	Cardiolipin control erucic acid 0.2 %	Cardiolipin treated erucic acid 9.8 %
14.0	—	0.3	—	0.2	—	0.4
16.0	16.7	8.5	7.7	5.9	1.7	2.1
16.1	0.4	0.9	—	0.2	0.2	0.5
18.0	30	26.3	36.1	25.1	3.7	6.2
18.1	10.4	11.7	8.6	14.4	10.5	9.0
18	14.3	17.[illegible]	6.1	11	78.0	55.7
18.3	—	0.5	—	0.4	0.1	1.6
20.0	0.3	0.5	0.4	0.4	0.1	0.8
20:1	0.4	4.4	0.5	6.9	0.3	4.1
20.2	0	1.2	0.3	0.7	0.6	1.4
20.4	4.0	20.5	22.8	15.3	2.8	4.0
22.1	—	5.3	—	7.8	—	10.8
22.6	3.1	2.7	17.5	11.5	2.0	3.3

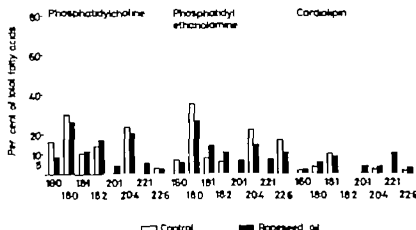


Fig 16. Relative distribution of total fatty acids of separated phospholipids in rat heart *homogenate* from rats fed diet of 40 Cal % fat containing 9.8 % erucic acid given as rapeseed oil for 10 days.

1 85 g trierucate per day for 5 days were influenced in the same way but to a less extent. The cardiolipin is found to contain 4 4 % erucic acid, and the linoleic acid content decrease from 82 % to 68 %. In the long-term feeding experiment (1 4 and 2.6 % erucic acid given as rapeseed oil for 28 days) there can be seen a small incorporation of erucic acid into the different phospholipids and to a slightly higher degree into cardiolipin. The incorporation of erucic acid into phosphatidylethanolamine and phosphatidylcholine caused a corresponding decrease in the arachidonic acid (C 20.4) content.

Discussion

The influence of rapeseed oil on the lipids of the homogenate and mitochondria of rat heart s given in Tables I—IV and Figs. 5—7 and 9. Rats treated with 9 8 % erucic acid in the diet show significant increase in the triglycerides of the heart mitochondria. This tendency is much less pronounced in rats treated with 1 4 or 2.6 % erucic acid.

The relative distribution of phosphatidylcholine, phosphatidylethanolamine and cardiolipin (diphosphatidyl glycerol) is given in Tables V—VI and Figs. 10—11. A similar composition is found in rat heart homogenates and in mitochondria. In rats treated with rapeseed oil there is a tendency to an increased concentration of phosphatidylcholine and decreased concentration of phosphatidylethanolamine. There is no change in the cardiolipin content.

The f tty acids of cholesterol esters, triglycerides and phospholipids of homogenate and mitochondria of rat heart are g en in T bles VII—XIV and Figs. 12—19. These results confirm and extend earlier observations (1 19) that erucic acid is predominantly incorporated in the heart triglycerides. The most interesting observation is that erucic acid seems to have a specific affinity to be incorporated into cardiolipin from rat heart mitochondria.

Since phospholipids are membrane constituents, knowledge about their distribution and metabolism may be important for a better understanding of the phenomena involved in lipidosis of heart and other diseases of the heart muscle. This information might also contribute to our knowledge of the importance of phospholipids in the ageing process of heart cells.

The incorporation of erucic acid into the cardiolipin molecule is of particular interest because cardiolipin is synthesized by the mitochondria and may be required for the

TABLE XI

Relative composition of total fatty acids of separated phospholipids in rat heart *mitochondria* from rats fed a diet containing 9.8 % erucic acid for 10 days.

Fatty acid	Phosphatidylcholine control erucic acid 0.[illegible] %	Phosphatidylcholine treated erucic acid 9.8 %	Phosphatidylethanolamine control erucic acid 0.[illegible] %	Phosphatidylethanolamine treated erucic acid 9.8 %	Cardiolipin control erucic acid 0 %	Cardiolipin treated erucic acid 9.8 %
14 0	0.1	0.1	0 1	1 6	0 1	0 1
16 0	14.9	15.5	9 7	8 9	1.[illegible]	15.9
16.1	0 1	0.8	0 1	2.2	0.6	0.3
18 0	36.0	26.6	33 9	16.5	1.5	5 7
18 1	9.5	14.3	8 1	13.3	10.2	8.2
18.2	1[illegible]1	18.6	6.4	14.3	81.9	47 1
18 3	—	0.6	—	0.8	0 1	1.3
20 0	0.2	0.4	0.3	—	—	—
20 1	0.3	4.5	0.3	4.8	0 4	4 7
20.2	0 1	1 1	0 1	1.0	0.9	1.2
20 4	23 7	11 7	[illegible]2.9	6 9	1.8	2.0
22.1	—	4 9	—	5.3	—	12.4
[illegible] 6	3.0	0.8	16.0	4 4	1.3	1 0

TABLE XII

Relative composition of total fatty acids of separated phospholipids in rat heart *homogenate* from rats fed a diet containing 1.4 % and [illegible] 6 % erucic acid for [illegible] days.

Fatty acid	Phosphatidylcholine control erucic acid 0 [illegible]	Phosphatidylcholine treated erucic acid 1 4 %	Phosphatidylcholine treated erucic acid 2.6 %	Phosphatidylethanolamine control erucic acid 0.2 %	Phosphatidylethanolamine treated erucic acid 1 4 %	Phosphatidylethanolamine treated erucic acid 2.6 %	Cardiolipin control erucic acid 0.[illegible] %	Cardiolipin treated erucic acid 1 4 %	Cardiolipin treated erucic acid [illegible]6 %
14 0	0	0 1	0 1	0 3	0 1	0.8	0 1	0.1	0.9
16 0	1[illegible]	1 1	12 4	7 7	7.0	7 0	0 9	1 0	5 7
16 1	0 3	0 5	0 3	0 8	0 1	0.3	0.1	0.6	1 0
17 0	0 3	0.[illegible]	0 3	0.[illegible]	0	0.3	0.1	0.1	0.9
18 0	[illegible]9 3	30 1	28 9	30 1	31	27.3	2 6	2.4	8.0
18 1	8 9	10 6	9 4	9 2	10 6	8.9	7	6 4	7.0
18 2	15 1	17 5	17.9	10.1	9 9	8 0	83.8	83.4	67.2
18 3	—	—	—	—	—	—	0 1	0.2	0.8
20 0	0	0	0 3	0.4	0 4	0.4	0.1	0.1	—
20 1	0 4	0 6	0 9	0 6	1 0	1.0	0 5	0.8	0 4
20.2	0 3	0 4	0.4	0.3	0 4	0 3	0 8	0.8	1.2
20 4	8 0	1 1	[illegible]6 0	28 4	25.8	27 4	2.6	[illegible].0	4.5
2[illegible] 1	0 4	0 6	1 0	0 4	0 9	1 3	0.1	0 7	0.8
2[illegible] 6	1 4	1 0	1	11 5	1[illegible]4	17.0	1.0	1 4	1.6

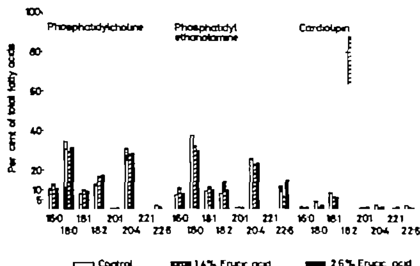


Fig 17 Relative distribution of total fatty acids of separated phospholipids in rat heart *mitochondria* from rats fed a diet of 40 Cal % fat containing 1 4 and 2.6 % erucic acid given as rapeseed oil for 28 days.

integrity of the inner membrane. Following the incorporation of erucic acid into cardiolipin there was a corresponding decrease in the content of linoleic acid in cardiolipin.

The molecular structure of fatty acids in lipid molecules has profound influence on the packing of these molecules in a bilayer (2_). In general, the longer the fatty acid, the more tightly packed are the molecules in a monolayer and the greater the unsaturation the more expanded the film. Thus at the same surface pressure, oleic acid forms more expanded film than erucic acid does, 48 resp 40 A /molecule. Erucic acid and other long chain monounsaturated fatty acids ha e physical characteristics like those of saturated fatty acids. Thus the incorporation of erucic acid into cardiolipin

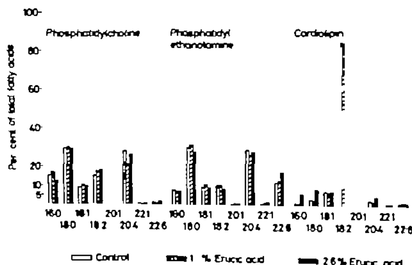


Fig 18 Relative distribution of total fatty acids of separated phospholipids *in rat heart homogenate* from rats fed a diet of 40 Cal % fat containing 1 4 and _6 % erucic acid given as rapeseed oil for 28 days.

TABLE XIII.

Relati e composiiton of total fatty acids of separated phospholipids in rat heart *mitochondria* from rats fed a diet containing 1.4 % and [illegible].6 % erucic acid for 28 days.

	Phosphatidylcholine			Phosphatidylethanolamine			Cardiolipin		
Fatty acid	control erucic acid 0.[illegible] %	treated erucic acid 1.4 %	treated erucic acid [illegible].6 %	control erucic acid 0.2 %	treated erucic acid 1.4 %	treated erucic acid 2.6 %	control erucic acid 0.[illegible] %	treated erucic acid 1.4 %	treated erucic acid 2.6 %
14.0	—	—	0.2	—	0.1	0.5	0.1	0.1	0.3
16.0	10.5	13.1	10.7	7.[illegible]	10.9	7.9	1.2	0.6	1.0
16.1	0.2	—	0.3	—	0.3	0.9	0.4	0.4	0.3
17.0	—	0.3	—	0.3	0.2	—	—	—	—
18.0	34.6	[illegible]9.6	31.5	37.[illegible]	3[illegible].0	30.1	4.0	1.0	1.9
18.1	7.9	9.9	9.1	9.3	11.[illegible]	10.0	8.5	6.4	6.3
18	1[illegible].7	16.7	17.[illegible]	8.0	14.0	9.6	79.4	87.4	85.2
18.3	—	—	0.1	—	—	—	0.1	0.[illegible]	0.3
20.0	0.3	0.1	0.3	0.4	0.[illegible]	0.3	0.1	—	—
20.1	0.4	0.4	0.9	0.5	0.8	1.0	0.6	0.6	0.9
20.[illegible]	0.[illegible]	0.4	0.3	0.[illegible]	0.6	0.5	1.0	0.7	0.7
20.4	30.9	8.1	[illegible]8.4	5.7	[illegible]2.8	[illegible]3.4	[illegible].4	1.1	1.3
2[illegible].1	0.[illegible]	—	0.8	0.1	0.5	1.0	0.1	0.6	1.3
2[illegible].6	[illegible].1	1.4	0.2	11.1	6.4	14.8	[illegible].1	0.9	0.5

TABLE XIV.

Relati composition of total fatty acid of separated phospholipids in rat heart *homogenate* from rats fed a diet containing 1.85 g trierucate per day given by tube for 5 days.

	Phosphatidylcholine		Phosphatidylethanolamine		Cardiolipin	
Fatty acid	control	treated erucic acid 1.85 g	control	treated erucic acid 1.85 g	control	treated erucic acid 1.85 g
14.0	0.2	0.2	0.2	0.1	0.2	1.0
16.0	1[illegible].7	14.3	9.7	7.1	1.1	6.8
16.1	0.6	0.7	0[illegible]	0.5	0.6	1.1
17.0	0.8	0.6	0.5	0.7	0.3	1.0
18.0	[illegible]	[illegible]9	[illegible]8.4	[illegible]6.9	[illegible]	4.3
18.1	10	10.4	6.0	7.7	7.[illegible]	9.0
18	1[illegible]	1[illegible].3	7.1	5.9	81.8	67.8
18.3	[illegible].1	0.1	—	0.1	0.2	0.7
[illegible]0.1	[illegible].3	0.7	0.[illegible]	0.7	0.[illegible]	1.6
[illegible]0	0.3	—	0.1	0.[illegible]	0.5	0.3
[illegible]0.4	[illegible]	1.1	2[illegible].0	[illegible]0.3	2.0	0.9
[illegible].1	[illegible].1	1.5	—	1.1	—	4.4
[illegible].6	10.[illegible]	9	[illegible]5.6	28.7	3.8	1.1

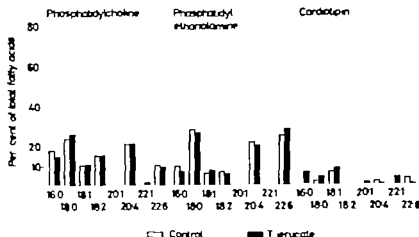


Fig 19 Relative distribution of total fatty acids of separated phospholipids in rat heart *homogenate* from rats fed a diet consisting of pellets with an addition of 1.85 g trierucate per day for 5 days.

and the corresponding decrease in the linoleic acid might influence the physical properties of this phospholipid characteristic of the mitochondrial inner membrane.

The role of polyunsaturated fatty acids in biological membranes has been very much discussed (21 28 29 30). It has been reported that in rats the replacement of fatty acids of the linoleate series with palmitoleate and oleate series, results in mitochondria which are more fragile during or after isolation (20 18).

The data presently available indicate a high degree of fatty acid selectivity of heart phospholipids particularly with regard to the synthesis of cardiolipin. There is the additional possibility of ester interchange which if extensive would mask any specificity or lack thereof, that existed during the initial synthesis of the phospholipids.

The mechanism by which this specific distribution of the erucic acid in membrane phospholipids is brought about remains to be established.

The existence of two pools of cardiolipin has been reported in rat liver mitochondria (3). One pool of cardiolipin is synthesized *de novo* and the other pool of cardiolipin which consists of only linoleic acid is synthesized by transacylation of the former. Erucic acid might be incorporated into cardiolipin in the same way as linoleic acid and qualitatively affect this pool. This is of great interest, because it has been found that cardiolipin is tightly bound to cytochrome oxidase (2), which indicates the importance of this phospholipid as a structural component of the respiratory chain.

It has also been shown that a certain proportion of unsaturated fatty acids are necessary in maintenance of mitochondrial function in a yeast mutant (15 23) unable to synthesize unsaturated fatty acids. Nearly complete loss of oxidative phosphorylation, respiratory control and valinomycin-dependent K+ uptake occured when the level of cellular unsaturated fatty acids fell below a certain minimum (23).

Reports from different laboratories (5 14) indicate that erucic acid inhibits the oxidation of other long-chain fatty acids in the mitochondria with an increased triglyceride synthesis in the heart tissue as a consequence. The specific effect of erucic acid on the cardiolipin with a decreased linoleic acid content might have a specific inhibitory effect on the mitochondrial fatty acid catabolism as well on the mitochondrial respiration and the energy supply of the heart.

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Studies of the Mode of Action of Erucic Acid on Heart Metabolism

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Abstract

The effects of erucic acid on the oxidative metabolism of rat-heart mitochondria have been investigated using intact animals, perfused beating heart, isolated mitochondria and mitochondrial extracts. Feeding rats with a diet containing erucic acid was found to lead to a diminished ability of the isolated heart mitochondria to oxidize various substrates, in accordance with previous reports (Houtsmuller *et al.*, Biochim. Biophys. Acta 218 (1970) 564). This effect was most pronounced with palmitylcarnitine as substrate, in which case the rate of oxidation was decreased by more than 50 % at such a low erucic acid content in the diet as 1.4 % given over 2—4 weeks. Oxidation of palmitylcarnitine was also found to be inhibited when erucylcarnitine was added to isolated heart mitochondria from control animals, in agreement with earlier observations (Christophersen and Bremer FEBS Lett. 23 (1972) 230; Biochim. Biophys. Acta 280 (1972) 506). The inhibition was accompanied by a decrease in the rate and extent of reduction of mitochondrial flavoprotein. Experiments with perfused beating rat-heart likewise revealed an inhibition of flavoprotein reduction, as well as nicotinamide nucleotide reduction, when erucate was added to the perfusing medium of the beating heart respiring with oleate — but not with octanoate — as substrate. These data together with those earlier published in the literature indicate that erucic acid may interfere with the enzyme system involved in the mitochondrial oxidation of long-chain fatty acids, probably at the level of acyl-CoA dehydrogenase. Kinetic data supporting this conclusion, obtained with extracts of rat-heart mitochondria containing the acyl-CoA dehydrogenase and electron-transferring flavoprotein system, are presented. The possible implications of these results for the known effect of dietary erucic acid in causing an accumulation of fat in the heart are discussed.

Experimental

When rats are fed a diet containing erucic acid, they show a striking accumulation of fat in the heart, as first shown by Abdellatif and Vles (1). Houtsmuller *et al.* (8) have shown that this accumulation of fat is accompanied by a decrease in the respiration of the heart mitochondria. We have investigated this effect in a series of experiments where the erucic acid content in the diet and the feeding time were varied. Different substrates were used to measure the respiration. These experiments confirmed the observations of Houtsmuller *et al.* (8) that the degree of inhibition was roughly proportional to the erucic acid content of the diet and that the inhibition diminished upon prolonged erucic acid feeding, as has also been shown the case with the fat accumulation. This phenomenon is illustrated in Table 1 which also shows that the respiration was significantly inhibited when palmityl carnitine (+malate) was used as a substrate, even at such a low erucic acid content in the diet as 1.4 cal-% at which dose respiration with Krebs-cycle intermediates or glutamate as substrates was hardly inhibited at all. The inhibition decreased after 8 weeks of erucic acid feeding.

TABLE 1

Respiratory rate (% of control) in the presence of various substrates in rat heart mitochondria from rats fed erucic acid 1—8 weeks ( ).

Erucic acid was added to the diet in the form of rapeseed oil with the corresponding amount of peanut oil added to the control diet. The diet consisted of 20 % casein, 53 % sucrose, 1 % oil (peanut or peanut + rapeseed), 1 % cellulose, salt and vitamines.

Mitochondria were isolated according to the method for preparing muscle mitochondria as described by Ernster and Nordenbrand (4). The method was slightly modified, in that sucrose was used as the homogenizing medium. Protein was determined with the biuret method (6). Respiration was measured polarographically with a Clark electrode. The mitochondria were incubated at 30 C in 3 ml of a medium consisting of KCl (80 mM), Tris-Cl (20 mM pH 7.4), P (5 mM pH 7.4) $MgCl_2$ (3 mM), EDTA (1 mM, pH 7.4).

Additions: pyruvate, glutamate or succinate (4 mM), malate (? mM), palmityl carnitine (30 µM), ADP (0.15 mM).

Substrate	Erucic acid g/100 g diet	1 w	2 w	4 w	8 w
Pyruvate + malate	1.4	99	100	110	103
	2.6	86	84	82	108
Glutamate + malate	1.4	78	66	85	86
	2.6	57	67	51	84
Succinate	1.4	1.4	95	119	118
	.6	64	89	107	103
Palmitylcarnitine + malate	1.4		46	45	68
	.6		35	13	80

The results described above indicated that erucic acid may primarily affect the oxidation of fatty acid. Support for this conclusion was obtained with isolated rat heart mitochondria. Table show that erucyl carnitine inhibited the oxidation of other long-chain acyl carnitines but not that of glutamate, pyruvate or succinate. Christophersen and Bremer (3) have reported similar result and concluded that the inhibition is not at the acyl-CoA-carnitine transferase reaction. It was therefore of interest to investigate the effect of erucyl carnitine on the first step of the β-oxidation of fatty acids, i.e. that catalyzed by the enzyme acyl-CoA dehydrogenase. This enzyme is a flavoprotein and its redox state can be followed in isolated mitochondria with a dual-wavelength spectrophotometer employing conditions as described by G. Lund *et al* (5).

The change in absorption at 475—510 nm was measured after preincubation of mitochondria with FCCP to facilitate the depletion of endogenous substrates, and then with antimycin A and rotenone, inhibiting the electron transport through the cytochrome system (Fig. 1). The addition of palmityl carnitine gave an extensive reduction of flavoprotein, indicative of a reduction of long-chain fatty acyl-CoA dehydrogenase. Erucyl carnitine gave a smaller reduction, which is evident when the changes of absorption in steady state are compared. This finding is in accordance with the observation that erucyl carnitine is oxidized at a slower rate than palmityl carnitine (cf. Table 1). Addition of palmityl carnitine and erucyl carnitine together or in sequence gave a change in absorbancy that was considerably smaller than that obtained with palmityl carnitine alone. These results clearly show that erucyl carnitine causes a diminished flavoprotein reduction with palmityl carnitine as substrate.

Kind cooperation by Professor B. Chance has enabled us to observe this effect also in the perfused heart. The redox states of both

TABLE 2.

Effect f erucyl car itin on th idation f acyl carnitines and other substrates in rat heart mitochondria.

Preparation and incubation of mitochondria, and respiration measurements as in Table 1 Additions: succinate, glutamate or pyruvate (5 mM), malat (2 mM) acyl carnitines (30 μM) Malate alone gave a rate of 37 nanoatoms oxygen/min/mg protein which has been subtracted

Substrate	Respiratory rate (nanoatoms O/min/mg protein) in the bsence of erucyl carnitine	in the presence of 30 μM erucyl carnitine
erucyl carnitine	—	40
palmityl carnitine	166	65
oleyl carnitine	124	87
glutamate	67	93
pyruvate	54	70
succinate	67	80

flavin and nicotinamide nucleotides were followed on the tissue surface of perfused hearts with a dual-wavelength fluorometer (2). The excitation and emission wa elengths were 366 and 480 nm, respectively for nicotinamide nucleotide, and 460 and 580 nm, respectively for the flavin Changes in fluorescence were recorded when the heart received pulses of fatty acids in the perfu sion medium. The fatty acids were added as the K-salts in a solution of fatty acid free albumin As shown in Fig. 2, repeated pulses of oleat gave parallel, cyclic changes in the redox state of the two components, the reoxidation obviously being due to ex haustion of the fatty acid. When a pulse of erucate was given between the pulses of oleate, the subsequent reduction with oleate was inhibited Repeated pulses of octanoate gave similar redox cycles to those found with palmitate, but these were unaffected by erucate.

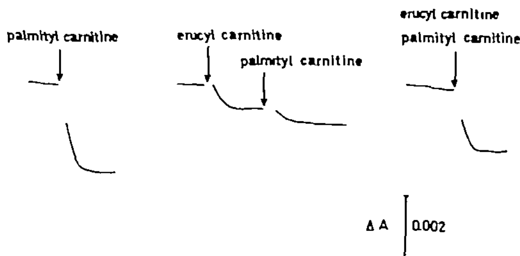


Fig. 1 *Reduction of flavoproteins in rat heart mitochondria.* Preparation of mitochondria and incubation medium as in Table 1 Mitochondria (2 mg protein/ml) were preincubated with FCCP (1 μM) for 3—4 minutes. Additions: Antimycin A (2.4 μg/ml), rotenone (1.5 μM), acyl carnitines (15 μM). Temp., 30° C.

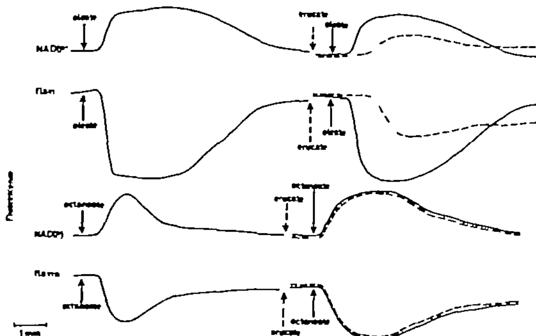


Fig. 2. *Reduction of flavin and nicotinamide nucleotides (NAD (P)) in perfused rat heart*

Rat hearts were perfused in an open Langendorff system (10). Pulses of oleate and erucate in 10 % solutions of fatty acid free bovine serum albumin and pulses of octanoate without albumin were injected into the perfusion medium. Final concentrations: oleate (0.5 mM), erucate (0.05 mM), octanoate (1 mM). Temp., 30 C.

The simplest interpretation of these results seemed to be that erucyl-CoA or one of its metabolites inhibits the flavoenzyme that is responsible for the first step of the β-oxidation of long-chain fatty acids. An attempt to investigate this possibility was made by using a soluble preparation from beef heart mitochondria containing fatty acid oxidizing flavoproteins. A procedure described by Holland *et al.* (7) was used, consisting of precipitation of the mitochondria with acetone, extraction of the acetone powder with phosphate buffer, precipitation of the extract with ammonium sulfate, and desalting on Sephadex G-25. The resulting extract contains a mixture of acyl-CoA dehydrogenases and electron transferring flavoprotein.

This extract catalyzed the oxidation of both palmityl-CoA and oleyl-CoA as well as erucyl-CoA in the presence of the artificial electron acceptors phenazine methosulfate (PMS) and 2,6-dichlorophenolindophenol (DCPIP). The reaction was followed by measuring the reduction of DCPIP at 600 nm. Fig. 3 illustrates the assay using palmityl-CoA as substrate and varying concentrations of enzyme. It may be seen that the reaction velocity was virtually linear with time and with enzyme concentration.

Fig. 4 compares the rates of DCPIP reduction at varying concentrations of palmityl-CoA, oleyl-CoA and erucyl-CoA (*solid lines*). As revealed by the double-reciprocal plots shown in Fig. 5 the three substrates gave approximately equal K_m values (4.6 μM) but somewhat different maximal velocities, palmityl-CoA being oxidized at the highest rate and erucyl-CoA at the lowest. Combination of either erucyl-CoA or oleyl-CoA with palmityl-CoA (Fig. 4 *dotted lines*) yielded reaction velocities that were intermediate between those obtained with either substrate alone.

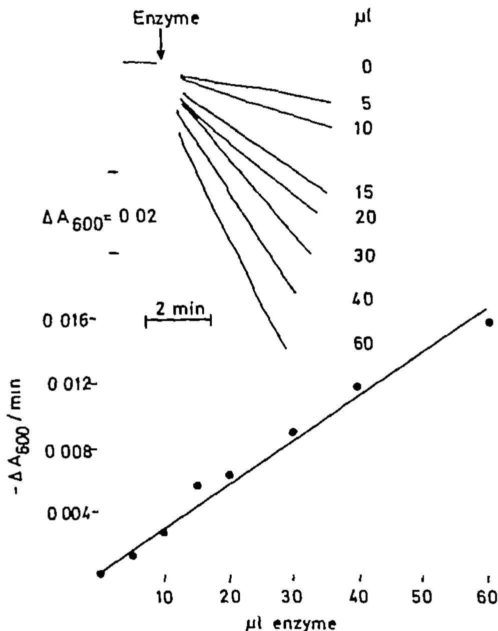


Fig. 3 *Assay of fatty acyl-CoA oxidation by enzymes extracted from beef-heart mitochondria*

Mitochondria were prepared as described by Löw and Wallin (9). The reaction mixture contained 133 mM K-phosphate, pH 6.8, 50 µM palmityl-CoA 0 8 mM PMS, 0.8 mM DCPIP and mitochondrial extract as indicated, in final volume of 1 ml. Temp 30° C.

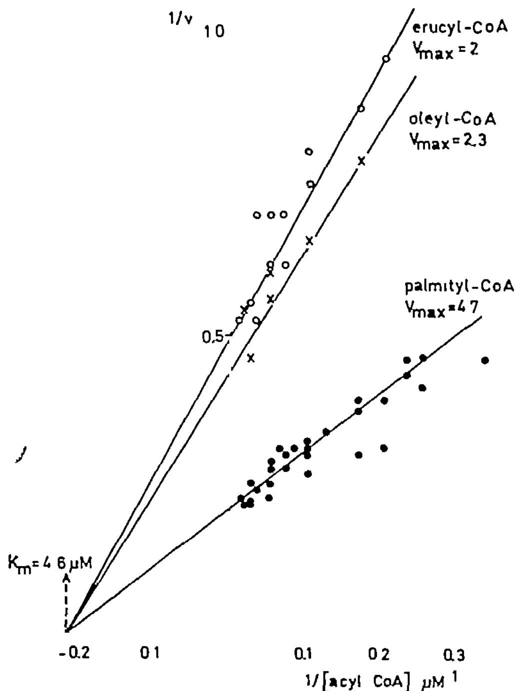


Fig. 5 *Double-reciprocal plots of the rates of DCPIP eduction at various concentrations of palmityl-CoA oleyl-CoA and erucyl-CoA* v is expressed in terms of nanomoles DCPIP reduced per min and 20 µl enzyme.

well as the less specific effects on mitochondrial respiration and thereby on the energy supply of the heart.

A more detailed knowledge of the fatty acid metabolizing system is necessary in order to further substantiate this conclusion and, in general, to understand the molecular basis of the pathological effects of erucic acid on the animal organism.

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General summary

Weanling rats were fed semisynthetic diets containing fat either as rapeseed oil or as arachis oil. The rapeseed oil contained erucic acid, which has been identified by gas chromatography as a C22.1 acid, in amounts varying from 0.3 % to 49 %. The control animals on the same basal diets had arachis oil (C22.1 0.1—1.-%) as the only fat source. The animals were studied with morphological, biochemical and physiological methods.

1 Feeding of rapeseed oil with a high content (49 %) of erucic acid, corresponding to 10 % of the diet (20 cal%) rapidly produced fatty accumulation of the heart muscle cells. After 20—40 days, in addition to the occurrence of f t droplets, lysis of the cells and infiltration of histiocytes were observed. In these experiments, 2 % (4 cal%) of erucic acid in the diet was the lowest level at which abnormal amounts of fat could still be detected in the myocardial cells by Collodial Scharlach Rot staining or electron microscopy. Although the abnormal fatty accumulation diminished on continued feeding of erucic acid, it was still present to a considerable degree after feeding for 160 days.

2. In all animals fed 10 % erucic acid i the diet for 160 days, multiple lesions, with fibrosis and cellular infiltration, were prevalent in all myocardial layers. In the animals given 2 % of erucic acid in the diet the lesions were few and serial sectioning had to be used to demonstrate them.

3 In one long term experimental series, heart lesions were also observed in serial sections among the controls fed arachis oil although to a much lesser extent than in the rats given 2 % of erucic acid in the diet. The individual lesion of the controls could not be distinguished from that found in the animals with an erucic acid consumption of 2 %. In another similar series of conventionally fed rats no heart lesions were found in the controls fed arachis oil, nor in the animals fed oil from the Canadian cultivar Oro, which gave a final erucic acid content of 0.06 % in the diet.

4 Germ-free animals were also fed semisynthetic diets containing rapeseed oil or arachis oil. Rats fed rapeseed oil from the Canadian cultivar Oro, which gave a final erucic acid content in the diet of 0.03 % showed no fibrotic lesions after long term feeding (80 days). The control animals on arachis oil were also free of lesions. Animals with high dietary levels of erucic acid (4.9 %) had extensive heart lesions.

5 The effects of erucic acid on the enzyme activities of isolated rat-heart mitochondria were investigated *i* *ivo* and *in vitro*. Feeding erucic acid (in the form of rapeseed oil) to rats resulted in diminished respiratory activity of the heart mitochondria, in agreement with earlier reports. This effect was most pronounced in tests with fatty acids as substrates. Erucic acid also inhibited the oxidation of long-chain fatty acids in perfused, beating rat heart, as revealed by measurements of flavine and nicotinamide nucleotide reduction *in situ*. Likewise, an inhibition of fatty acid oxidation by erucic acid was observed in isolated rat-heart mitochondria *in vitro* thus confirming recent data in the literature. Preliminary results from studies with enzyme extracts from heart mitochondria indicate that the inhibition of fatty acid oxidation by erucic acid is localized to the first oxidation step of the β-oxidation sequence, i.e. reaction catalyzed by the acyl-CoA dehydrogenase flavoprotein involved in the oxidation of long-chain fatty acids. These findings are now being followed up with the purified enzyme. In conclusion, these results provide the biochemical background to the accumulation of fat in heart tissue caused by orally administered erucic acid.

6. The fatty acid composition of the main membrane phospholipids of rat heart mitochondria, i.e. phosphatidylcholine, phosphatidylethanolamine and cardiolipin, was influenced by the orally administered erucic acid. The erucic acid seemed to have a spe

cific affinity for cardiolipin and its incorporation into the cardiolipin was followed by a corresponding decrease of linoleic acid. This is an important observation since cardiolipin is a component of the inner membrane of mitochondria and its high affinity for erucic acid might influence the normal function of heart mitochondria.

7. The animals with the highest erucic acid consumption (10 % of the diet) and the most extensive lipidosis had normal electrocardiograms.

8. The lowest level of erucic acid in the diet to result in demonstrable changes of the myocardium in these animal studies was 2 %. This is equivalent to 4 % of the energy intake. Before the reduction of erucic acid in consumer margarine decided upon in 1970, the mean consumption of erucic acid in Sweden was calculated to be 3—4 % of the energy intake. The present mean consumption might be estimated to be 0.4 % of the energy intake.

9. The present studies have confirmed and extended earlier findings on the effects of erucic acid in rats and have also introduced additional evidence of basic effects on the heart, such as impaired energy metabolism and mitochondrial membrane interference. They demonstrate that fatty accumulation in myocardial cells is not a transient phenomenon, since some lipoidosis persists throughout the entire experimental period (160 days) even when low amounts of erucic acid are fed. Although there is no direct evidence at present time of any similar effects of erucic acid consumption in man, the persistence of the fatty accumulation and the fact that effects on the heart have been demonstrated in all tested animal species fed rapeseed oil high in erucic acid call for great caution in using products containing these long-chain fatty acids as significant sources of fat in human or animal nutrition.

Acta Medica Scandinavica

Supplementum 586

Swedish Co–operative CCU Study

A study of 2008 patients with acute myocardial infarction from twelve Swedish hospitals with coronary care unit

Part I A description of the early stage

By Rune Henning and Torbjorn Lundman

Part II The short-term prognosis

By Rune Henning

Distributed by The Almqvist & Wiksell Periodical Company Stockholm, Sweden

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PART I

A description of early stage

The study was initiated by the Swedish Society of Cardiology
The study was coordinated by H Eliasch
The computer analysis was done by T Lundman.
The statistics were done by R. Henning.
The report was written by R. Henning and T Lundman.
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Aided by grants from the Swedish National Association against Heart and Chest Diseases.

CONTENTS

Part I The early stage of AMI

ABBREVIATIONS

A. flutter	Atrial flutter
AF	Atrial fibrillation
AMI	Acute myocardial infarction
AP	Angina pectoris
aVF aVL, aVR	ECG leads conventional augmented Wilson leads
A–V	Atrioventricular
A–V block I	First degree A–V block
A–V block II	Second degree A–V block
A–V block III	Third degree A–V block or complete heart block
BP	Systemic arterial blood pressure
CCU	Coronary care unit
CHD	Coronary heart disease
CHF	Congestive heart failure
CR_{1-7}	ECG leads conventional chest position against right arm
ECG	Electrocardiogram
IHD	Ischaemic heart disease
LBBB	Left bundle branch block
LHF	Left heart failure
MI	Myocardial infarction
NR	Nodal rhythm
POe	Pulmonary oedema
RBBB	Right bundle branch block
RHF	Right heart failure
SGOT	Serum glutamate oxaloacetate transaminase
SGPT	Serum glutamate pyruvate transaminase
SVB	Supraventricular bradycardia
SVEB	Supraventricular ectopic beat
SVT	Supraventricular tachycardia
VEB	Ventricular ectopic beat
VF	Ventricular fibrillation
VT	Ventricular tachycardia

Hospital mortality in acute myocardial infarction has been very high and relatively constant for many years. In many reports from different parts of the world the rate amounts to about 30–40 per cent (Master et al. 1939 Rosenbaum and Levine 1941 Billings et al. 1949 Wållgren 1950 Lindén 1952, Biörck et al. 1957 Honey and Truelove 1957 Harnagel et al. 1959 Brown et al. 1963 *Hughes et al. 1963 Sievers 1963 Wahlberg 1963* Lown et al. 1967 a, McMillan et al. 1967 Baily and Beaven 1968 Day 1968, Killip and Kimball 1968, Norris et al. 1968, WHO Report 1968 McDonald et al 1969 Isacsson et al. 1969 Chapman 1970 Christiansen et al. 1971). Higher rates have been reported in some works (Levine 1929 Clark 1933 Ejrup and Nylin 1943 White et al. 1960) and lower in others (Conner and Holt 1930, Howard 1934 Doscher and Poindexter 1950, Smith and Denham 1951 Beard et al. 1960 Hipp et al. 1961) The wide range of mortality rates may be explained by differences in the composition of the materials in terms of mean age diagnostic criteria, preselection of patients, indications for admission, type and periods of care

Over the years various methods of treatment have been developed with a view to reducing the high mortality in acute myocardial infarction. The first clinically successful termination of ventricular fibrillation in man was reported in 1947 by Beck et al. who used emergency thoracotomy direct cardial massage and applications of alternating-current countershock to the exposed heart. Zoll et al. showed in 1956 that ventricular fibrillation in man could be terminated by electric countershock with the electrodes applied extrathoracically In 1960 Kouwenhoven et al. developed the technique of external cardial compression with ventilation, by which circulation may be restored immediately in an emergency/or cardiac arrest, thereby avoiding ischaemic injuries in organs of vital importance. Cardioversion was introduced by Lown et al. in 1962 for terminating cardiac tachyarrhythmias, an electric shock being applied externally in a manner which was shown to be both rapid and safe. In 1952 Zoll was the first to apply the method of external electric stimulation for resuscitation of the human heart in ventricular standstill and in 1959 Hunter was the first to achieve long-term pacing in a patient with Adams-Stokes disease Special hospital departments – coronary care units (CCUs) – for patients with acute myocardial infarction were started in 1962 (Brown et al. 1963 Day 1965 Meltzer and Kitchell 1966).

The development of electrical equipment and instruments, permitting continuous long-term electrocardiographic monitoring, facilitated the immediate discovery and rapid treatment of ventricular fibrillation and asystole (Brown et al. 1963 Day 1965 Julian et al. 1964 Robinson et al. 1964) Continuous ECG-registration disclosed that 80–90 % of all patients with acute myocardial infarction have arrhythmias of some kind (Julian et al. 1964 Goble et al. 1966 Fluck et al. 1967 Lawrie et al. 1967 Lown et al. 1967 a, Killip and Kimball 1968 Mogensen 1970).

Earlier reports on the total incidence of arrhythmias *gave rates between 15 and 70 % (Master et al.* 1937 Rosenbaum and Levine 1941 Mintz and *Katz 1947 Smith* and Denham 1951 Ball et al. 1955 Johnson and Minor 1958, Scherf 1958 Imperial et al. 1960). Continuous ECG-registration and careful observation gave greater insight into the frequency of different arrhythmias and their prognostic importance This has facilitated increased possibilities to prophylactic treatment of some deleterious arrhythmias because these often are preceded by less serious arrhythmias and possibly led to some reduction of so-called primary arrhythmias, it means unexpected ventricular *fibrillation and cardiac standstill (Lown et al.* 1967 a, Killip and Kimball 1968).

The reduction of hospital mortality from myocardial infarction which Goble and co-workers (1966) reported after the introduction of a coronary care unit was followed by similar results elsewhere (Day 1965 Fluck et al. 1967 Killip and Kimball 1967 Lawrie et al. 1967 Lown et al. 1967 a Restieaux et al. 1967 Wallace et al. 1967 Marshall et al. 1968, Meltzer 1968 Sloman et al. 1968, Thomas et al. 1968) In most of these

studies, the results of coronary care are compared with earlier mortality figures from the literature or from earlier patient series at the same hospital. Such comparisons may lead to faulty conclusions as the mortality may vary during different periods at the same hospital (Schnur 1953 a, Grace 1967) and so may the composition of the materials, especially the mean age, variations in diagnostic criteria, preselection of patients and indications for admission (Klaus et al. 1970).

Few direct comparisons have been made between two comparable patient groups with acute myocardial infarction treated at the same hospital *during the same period and with the same diagnostic criteria*, one being warded at CCU and the other at general medical wards (Killip and Kimball 1968). Two such controlled studies, one at Serafimer Hospital, Stockholm (Hofvendahl 1971) and *one at Municipal Hospital, Copenhagen* (Christiansen et al. 1971) showed that compared with the controls treated in conventional wards, mortality in the CCU group was reduced by about 50 per cent. The reduction of mortality in acute myocardial infarction is probably achieved in particular by an active antiarrhythmic treatment. In contrast treatment of severe heart failure, frank pulmonary oedema and shock after infarction has proved very disappointing. The mortality in patients with these serious conditions is still very high even if the patients are treated in coronary care units (Goble et al. 1966, Killip and Kimball 1967, Lawrie et al. 1967, Lown et al. 1967 a, Meltzer 1968, Sloman et al. 1968, Norris et al. 1969 a, b, Bloomfield et al. 1970, Scheidt et al. 1970, Sjögren 1970, Sloman and Brown 1970, Hofvendahl 1971, Nyquist 1972).

PLANNING THE STUDY

Only limited experience of CCUs in Swedish hospitals was available in 1968. A substantial material was reported from the Hospital in Borås (Wingstrand 1967, Isacsson et al. 1968) but materials from other hospitals were not yet available for further analyses (Bergquist et al. 1968, Biörck et al. 1969, Lundman et al 1969). In view of the fact that various types of CCUs were to be planned or started up at several Swedish hospitals during the next few years, in 1968 the Swedish Society of Cardiology initiated a major co-operative study of CCU activity.

The study aimed in particular at providing a detailed picture of patients during the early phase of acute myocardial infarction. The creation of CCUs involves an expansion of emergency admissions, with all the personnel and material that this entails, and it was considered that such a study could be helpful to indicate how a CCU could be organized to the best advantage.

The study covered the whole year of 1969 and twelve hospitals took part, most of them having started their CCU the year before. A brief description of the hospitals is given below. In order to make the material as uniform as possible, some rules were set up for the selection of patients, diagnostic criteria, registration of patients and certain principles of treatment. No changes were to be instituted in the organization and general policy during the year of the study. The results were to be worked up for all hospitals conjointly.

Criteria for admission

In general the following criteria for admission to CCU were applied by all hospitals.

1 Central chest pain of more than 15 min. duration and with onset during the last 48 hours.
2 Frank pulmonary oedema without previously known valvular heart disease
3 Shock without suspicion of acute hypovolemia, bleeding or intoxication
4 Life threatening arrhythmias (ventricular tachycardia ventricular fibrillation asystole and atrioventricular block III)

A shortage of beds obliged some hospitals to apply an upper age limit Sahlgrenska Hospital 70 years, (Henning and Holmberg 1971) Malmö Community 70 years in the event of a bed shortage Karolinska Hospital and Falu County Hospital 80 years (Ahlmark et al. 1971)

Diagnostic criteria

The diagnostic criteria based on daily ECG records and serum-enzyme determinations and findings at autopsy were as follows

I Myocardial infarction (MI)
Either two of criteria a b and c or criterion d should be fulfilled.
a) *central chest pain, pulmonary oedema, syncope or shock.*
b) appearance of a pathological Q-wave and or appearance or disappearance of a localized ST-elevation followed by a T-inversion in two or more of the twelve leads.
c) Two SGOT-values of 40 units or more with a maximum about 4 hours after onset of symptoms in combination with a SGPT maximum after about 36 hours and lower than the SGOT maximum
d) Findings at autopsy of myocardial necrosis of an age corresponding to the onset of symptoms

II Suspected myocardial infarction Criterion a) fulfilled in combination with partial fulfillment of criteria b) and/or c) *e.g. appearance of a localized T-inversion or bundle branch* block or increase of only one SGOT or SGPT-value

III. Observation case Criterion a) fulfilled but not b), c) or d).

In the following analysis, patients with myocardial infarction are grouped according to the number of previous infarctions none one two or more

Definitions

I PREVIOUS DISEASES

Angina pectoris (AP)

1 Substernal pain with or without radiation.
2. *Left-side* precordial *chest pain* with radiation in the *left* arm and which (concerning both alternatives) *commencing during effort* and *disappearing within ten min. when the patient* stops or takes nitroglycerine

Angina pectoris has been grouped by duration less than 1 month, 1 6 months, more than 6 months (chronic angina) and uncertain

Congestive heart failure (CHF)

Symptoms of CHF (e.g. dyspnoea dependent oedema, cough during effort or at night) which have been treated with digitalis and/or diuretics.

Hypertension

Treatment with antihypertensive drugs, or a physician has told the patient that the blood pressure was high.

II PHYSICAL FINDINGS

Disturbances in consciousness imply that the patients were mentally affected (listless confused not orientated in time and/or space Loss of consciousness implies that any contact with the patient was not possible

Left heart failure (LHF)

The appearance of a third heart sound and/or the presence of basal rales and/or central vascular enlargement on chest X-ray

Frank pulmonary oedema (POe)

Patients with rales heard all over the chest in association with frothy sputum. (Those classified in this group were not included under LHF.)

Right heart failure (RHF)

Enlarged, tender liver and/or distended veins of the neck in sitting position and/or a central vein pressure of 13 cm water or higher

Hypotension

Systolic blood pressure of 90 mm Hg or lower without clinical signs of shock.

Shock

Systolic blood pressure below 90 mm Hg in combination with impaired peripheral circulation as confusion, anxiety, pallor, cyanosis, peripheral chilliness, cold sweat and oliguria. (Those classified in this group were not included under hypotension.)

III. ARRHYTHMIAS

Normal sinus rhythm was characterized by regular P-QRS-complex with a PQ-time of 0.12–0.22 seconds and a QRS-duration of 0.10 seconds or less at a frequency of 50 to 100 beats a minute. All other ECG findings showing some disturbance of rate, rhythm or conduction resulted in a diagnosis of arrhythmia.

First degree A–V block (A–V block I)

PQ-intervals of more than 0.22 seconds at normal frequency

Second degree A–V block (A–V block II)

Regular P-wave appears intermittently without a subsequent conducted QRS-complex.

Third degree A–V block (A–V block III)

P rate exceeds a stable and independent QRS-rate usually below 50 beats per minute

Left bundle branch block (LBBB)

QRS-duration of 0.11 seconds or more in combination with typical configuration on ECG-recording.

Right bundle branch block (RBBB)

QRS-duration more than 0.11 seconds in combination with typical configuration on ECG-recording.

Supraventricular tachycardia (SVT)

Frequency over 100 beats per minute and a positive P wave in leads I and II.

Supraventricular bradycardia (SVB)

Frequency below 50 beats per minute and a positive P-wave in leads I and II.

Conventional criteria were used for *supraventricular extra systolic beats (SVEB), atrial flutter (A. flutter) and atrial fibrillation (A. F), sinus arrest and nodal rhythm. (Lundman et al. 1968 a).*

Ventricular ectopic beats (VEB)

Premature QRS-complex with a duration of more than 0.10 seconds and a configuration different from the regular QRS-complex. VEB was grouped by frequency into none, occasional, 1–5 per minute and more than 5 per minute, and by type as monofocal, multifocal (i.e. with different QRS-configuration), paired (i.e. 2 VEB in succession) and VEB of the R on T type.

Ventricular tachycardia (VT)

A rhythm with 3 or more VEB in succession.

Ventricular fibrillation (VF)

Fast, irregular oscillations with varying amplitudes, without similarity to QRS complexes and followed clinically by signs of circulatory arrest.

Ventricular standstill (asystole)

RR-interval of more than 3 seconds.

VF and asystole were grouped according to Olivier et al (1967) primary VF and asystole when these arrhythmias occurred unexpectedly without signs of cardial failure and/or shock, secondary VF and asystole when these arrhythmias complicated pre-existing cardiac failure and/or shock

These criteria of arrhythmias are those generally applied in CCU by the surveillance personnel to whom the arrhythmias were explained and defined with the help of an illustrated manual (Lundman et al. 1968 a)

It should be pointed out that on occasions the usual ECG-recordings had to be supplemented with more subtle analyses in order to arrive at the appropriate diagnosis. Even then the interpretation sometimes involved an element of uncertainty in which case the most probable arrhythmia was registered. The differentiation of VEB from SVEB with aberrant conduction was suggested by a similar initial QRS-deflection to the regular QRS-complex and a short RR interval following a long one favouring aberration.

Electrocardiography

Routine 1 lead ECGs including leads I II III aVR, aVL, aVF CR_1 2 4 5 6 and 7 were registrated upon admission and then daily for at least the next three days Supplementary examinations using oesophagus or right atrial electrodes, were occasionally performed for further differential diagnosis between arrhythmias

The site of infarction judged from the changes observed in the daily ECG-recordings at the CCU was coded as negative anterior lateral inferior antero-lateral antero-inferior infero-lateral antero-lateral inferior and uncertain Anterior wall infarction was judged from changes in two or more of leads I and CR_1 CR_4 lateral wall infarction from changes in leads II CR_5 CR_7 inferior wall infarction from changes in leads AVF II III and reciproce ST-depression over anterior wall By combined infarction is meant changes over anterior and lateral wall and/or inferior and lateral wall simultaneously Some of these widespread ECG-changes may be due to subendocardial infarction and some to secondary pericarditis.

Serum-enzyme determinations

Blood for enzyme determination was taken on admission and in the morning of at least the next three days. Serum glutamate oxaloacetate transaminase (SGOT) was determined by the direct method described by Karmen Wroblewski and La Duc (1955) the normal value was below 40 units per ml

Data registration

All observations in the CCU e.g. physical findings and occurrence of arrhythmias, were registered on a special sheet day by day The notes from these sheets and other data (e.g. history of previous diseases physical activity smoking habits symptoms at onset laboratory findings stay in CCU and at hospital and findings at autopsy) were entered on a special data form (Lundman et al 1968 b) The history and clinical findings on admission were filled in by the physician, the laboratory data and arrhythmias generally by the nurses. The data form was checked before the patient left the CCU When the patient was discharged from the hospital the form was completed and checked again as soon as possible

All information was coded directly on the form and transferred to punched cards The statistics were processed mainly with computer programs for cross tabulation Before the analyses the data were controlled in various ways with the use of a computer to avoid missing and unrealistic data

Statistical methods

The Chi-square test was used for testing the significance of differences between relative numbers Degrees of significance were tested at the 5 1 and 0 1 per cent level In the text this is expressed as not significant ($p>0.05$) probably significant ($p<0.05$) significant ($p<0.01$) and highly significant ($p<0.001$) (Scientific Tables, Documenta Geigy 1970)

HOSPITALS PARTICIPATING

1 County Central Hospital, Borås.
Serves a population of about 125.000 individuals of whom 70 000 live in the city and 55.000 in the country. The Dept. of Medicine has 148 beds. The CCU is part of a medical intensive ward of 14 beds. Six beds are equipped with bedside oscilloscope and there is a 6-channel oscilloscope in the central area. Another 3 beds in the ward can be ECG-supervised. There is no special after-care ward.

2. County Central Hospital Falun.
Serves a population of 146 000 individuals, of whom 75 000 live in the town and 71.000 in the country. The Dept of Medicine has 145 beds. The CCU is part of a medical and surgical intensive ward totalling 21 beds. Normally 4 beds are used for patients with heart diseases and another two beds can be used when necessary. There are 6 bedside oscilloscopes and a 4-channel slave oscilloscope is placed in the central area. There is no special after-care ward.

3. Karolinska Hospital Stockholm
A university hospital situated in the north of Stockholm, serving an undefined population. The Dept. of Medicine has 100 beds. The CCU has 4 beds, all of which are equipped with bedside oscilloscope. A 4-channel slave oscilloscope is placed in the central area. A special after-care ward with 12 beds can be enlarged to 15 beds if required and caters for all patients with acute myocardial infarction during the rest of their hospital stay.

4 Regional Hospital, Linköping.
A university hospital serving an undefined population. The Dept of Medicine has 172 beds. The CCU is part of a cardiology ward of 29 beds. Four beds are equipped with bedside oscilloscope and there is a 4-channel oscilloscope in a central area without direct observation of these 4 beds in the CCU. All other beds in this ward are used for after-care of the patients for the rest of their hospital stay.

5 General Hospital, Malmö.
A university hospital serving a population of about 255 000 individual. The Dept. of Medicine has 234 beds. The CCU is part of a special ward of 26 beds for patients with heart diseases. Four beds are equipped with bedside oscilloscope and a 4-channel oscilloscope is placed in a central area. Twenty of the beds in the ward are used for after-care and patients with acute myocardial infarction are treated there for the rest of their hospital stay.

6. Sahlgrenska Hospital, Göteborg.
A university hospital serving a population of about 445.000 individuals. There are five Dept.s of Medicine with 489 beds all told. The CCU is part of a special ward with 24 beds for patients with heart diseases. Six beds are equipped with bedside oscilloscope and a 4-channel slave oscilloscope is placed in a central area in the CCU. On leaving the CCU the patients are treated for another five days in the special ward before being discharged to general wards.

7 Seraphimer Hospital, Stockholm.
A university hospital situated in central Stockholm, serving at the time of the study an undefined population within Greater Stockholm. The Dept. of Medicine has 191 beds. The CCU has 7 beds with bedside oscilloscope in every room and two 4-channel oscilloscopes in the central station. There is an adjacent after-care ward with 14 beds. Elective beds can be equipped with bedside oscilloscope connected to the central station in the CCU. All patients with large myocardial infarction or with complication were discharged to this after care ward, where they were treated during the rest of their hospital stay. All other patients, a minority received after-care in general wards.

8. S:t Görans Hospital Stockholm.
Also located in the central part of Stockholm and serving an undefined area. There are two Dept.s of Medicine with 200 beds all told. The CCU with 4 beds, is part of a general medical ward of 14 beds. Two of these four beds, which are used for

patients with acute myocardial infarction are equipped with bedside oscilloscope. There is no special after-care ward

9 County Central Hospital Uddevalla.
Serves a population of 125 000 individuals of whom 50.000 live in the town and 75.000 in the country The Dept. of Medicine has 118 beds. The CCU consists of 4 beds. all equipped with bedside oscilloscope A 4-channel oscilloscope is placed in the central area An after-care with 24 beds is directly connected to the CCU and two of its beds can be equipped with bedside oscilloscope All patients with acute myocardial infarction are discharged to the after-care ward for the rest of their hospital stay

10. University Hospital Uppsala
A university hospital serving a population of about 200.000 individuals of whom 115 000 live in the city and 85 000 in the country The Dept of Medicine has 170 beds. The CCU is part of a ward with 26 beds for patients with heart diseases. 4 beds are equipped with bedside oscilloscope and there is a 3-channel oscilloscope in the central area There is no special after-care ward.

11 County Central Hospital Vänersborg
Serves an undefined population. The Dept. of Medicine has 136 beds. The CCU is part of a medical and surgical intensive ward with 14 beds all told. Four beds are equipped with bedside oscilloscope and there is a central oscilloscope with one channel in the central area. No special after-care ward

12. County Central Hospital Östersund
Serves a population of about 126 000 individuals, of whom 27 000 live in the town and 99 000 in the country The Dept. of Medicine has 145 beds. The CCU is part of an intensive ward with 25 beds. Seven beds are used for patients with heart diseases. Five beds are equipped with bedside oscilloscope and a 4-channel oscilloscope is placed in the central area. Regular ECG recordings are made every half-hour on every patient with acute myocardial infarction No special after-care ward.

Fig. 1 shows the location of the participating hospitals, the numbers correspond to those in the text above

Twelve hospitals participated in the study and the complete material consists of 2008 patients, who were treated in the respective CCUs during 1969 with the diagnosis of acute myocardial infarction. 1447 were men (72.1%) and 561 women (27.9%) giving a male/female quotient of 2.6. The mean age of all the patients was 65.5 years, for men 63.8 and for women 69.8 years. The number of patients from each hospital is given in Table 1 together with their mean ages totally and by sex. The table also shows the age distribution by decades in per cent of the total number of patients from each hospital.

It will be seen that the mean age for Sahlgrenska Hospital is definitely lower than for the other hospitals. This is explained by the selection criteria for this hospital, with an upper age limit of 70 years (Henning and Holmberg 1971). The few patients, over 70 who nevertheless received primary treatment in the CCU there were admitted on the indication of "life-threatening arrhythmias". The mean age for General Hospital in Malmo was also of the same reason lower but if there was place available even older patients were admitted to the CCU.

Fig. 2 shows the age and sex distribution in five-years classes. The form of the age-distribution curve is rather similar to that of the normal distribution (in spite of an upper age limit for primary treatment in some CCUs (Sahlgrenska and Malmo 70 years, Falun and Karolinska 80 years). The figure also shows that men predominate in the younger age groups and that the sex difference does not level out until after 70 years of age: higher age groups have about equal numbers of men and women.

Elderly patients make up a large part of the material from the hospital in Uddevalla and the same is true to a lesser extent of the hospitals in Borås and Falun. Such differences in the composition by age must be taken into account when comparing different hospitals, especially concerning the frequency of complications and the mortality.

It could also be anticipated that other differences existed between different types of hospitals.

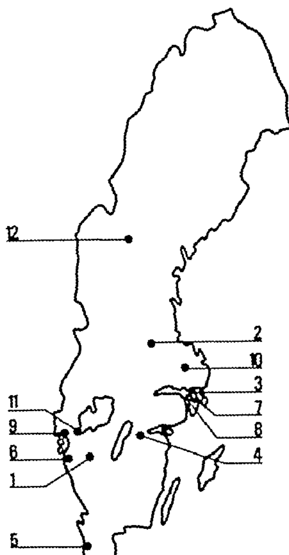


FIGURE 1. The localization in Sweden of participating hospitals. Numbering refers to the description of the hospitals on page 13–14.

To illustrate this a comparison has been made between 906 patients from five hospitals (nos 3, 5, 6, 7 and 8) in three big cities (Stockholm, Gothenburg and Malmö) and 1102 patients from seven county hospitals (nos 1, 2, 4, 9, 10, 11 and 12). The differences were remarkably small and only significant for age (higher mean age in county hospitals), for smoking habits (more smokers in

patients with acute myocardial infarction, are equipped with bedside oscilloscope. There is no special after-care ward.

9 County Central Hospital, Uddevalla.
Serves a population of 125.000 individuals, of whom 50 000 live in the town and 75.000 in the country The Dept. of Medicine has 118 beds. The CCU consists of 4 beds, all equipped with bedside oscilloscope. A 4-channel oscilloscope is placed in the central area. An after-care with 24 beds is directly connected to the CCU and two of its beds can be equipped with bedside oscilloscope All patients with acute myocardial infarction are discharged to the after-care ward for the rest of their hospital stay

10. University Hospital, Uppsala
A university hospital serving a population of about 200 000 individuals, of whom 115 000 live in the city and 85 000 in the country The Dept. of Medicine has 170 beds. The CCU is part of a ward with 26 beds for patients with heart diseases. 4 beds are equipped with bedside oscilloscope and there is a 3-channel oscilloscope in the central area. There is no special after-care ward.

11 County Central Hospital, Vanersborg.
Serves an undefined population. The Dept. of Medicine has 136 beds. The CCU is part of a medical and surgical intensive ward with 14 beds all told. Four beds are equipped with bedside oscilloscope and there is a central oscilloscope with one channel in the central area. No special after-care ward.

12. County Central Hospital, Östersund
Serves a population of about 126 000 individuals, of whom 27 000 live in the town and 99 000 in the country The Dept of Medicine has 145 beds. The CCU is part of an intensive ward with 25 beds. Seven beds are used for patients with heart diseases. Five beds are equipped with bedside oscilloscope and a 4-channel oscilloscope is placed in the central area. Regular ECG-recordings are made every half-hour on every patient with acute myocardial infarction No special after-care ward.

Fig. 1 shows the location of the participating hospitals, the numbers correspond to those in the text above.

RESULTS

Introduction

The result were analysed here from various points of view with the emphasis on the variables listed below which seemed to be of particular importance of interest in the description of the early stage of acute myocardial infarction. The large amounts of descriptive data provide a detailed picture of the early stage of patients with acute myocardial infarction, especially during the first day in CCU

Variables analysed
1 Age factor
2 Sex
3 Previous diseases
4 Delay
5 Physical findings
6 Site of infarction
7 Arrhythmias

The variables have been broken down into the *groups which feature as column headings in the* tables. The sum of the patients in each group has been used as the base for calculating percentages.

Age has been analysed against most variables, including previous diseases delay smoking habits, clinical findings on admission and during the first day in CCU ECG-recorded arrhythmias on admission and during the first day the localization of infarction, maximum SGOT and medicamental therapy during the first day The distribution by age and the mortality in different age-groups are given.

Age distribution

The percentage distribution of the patients by decades is given in Table 1 which shows that patients in the 7th decade dominate in this material (36.3 per cent).

Mortality

The age-related mortality in CCU and while in hospital is presented for the various hospitals in Table 2 and fig. 3 shows that there is a clear trend for mortality to rise with age especially after 65

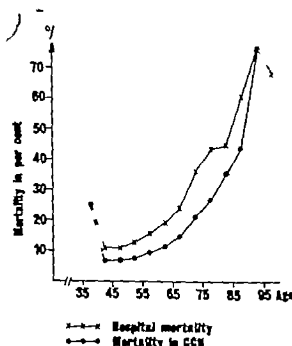


FIGURE 3 Mortality in CCU and during hospital stay in relation to age.

Comments

High age has been identified as a negative factor by most authors (*Master et al* 1939 Rosenbaum and Levine 1941 Woods and Barnes 1942, Chambers 1946 Lindén 1952 Biorck et al 1957 Honey and Truelove 1957 Ikkala and Kalpainen 1957 Harnagel et al 1959 Peel et al. 1962, Hughes et al. 1963 Wahlberg 1963 Pell and D Alonzo 1964 Lemlich 1965 Bailey and Beaven 1968 Norris et al. 1969 b Sloman and Brown 1970 Thompson and Sloman 1971).

The present findings clearly show the great association of age on the short term prognosis (*while in hospital in general less than 8 weeks*). According to fig. 3 mortality rises more steeply after the age of 65 years. This agrees with what *several other authors have found* (*Master et al.* 1939 Lindén 1952 Ikkala and Kalpainen 1957 Beard et al. 1960 Thould 1965 Bailey and Beaven 1968 *Bullock et al. 1970 Nielsen 1970*). The present study disclosed a high mortality among young men and this, too has been reported by *other authors* (*Yater et al.* 1951 *Biörck et al.* 1957 Sloman and Brown 1970) but the number of patients in the younger age groups is too small *to allow any conclusions.*

Previous diseases

Some previous diseases are listed in relation to age *in Table 3*

Angina pectoris

There were no significant differences between different age groups concerning *chest pain lasting* either less than one month or 1–6 months. There is a significant increase in frequence of chronic angina (*chest pain more than six months*) *up to* the 7th decade.

Previous myocardial infarction

There were *no significant differences in the frequence* of previous myocardial infarction between different age-groups.

TABLE 2. Mortality by age during stay in CCU and total stay in hospital, totally and for each hospital.

Patients			Age						
Mortality		Total	≤39	40–49	50–59	60–69	70–79	80–89	≥90
Total material	n	2008	10	137	404	779	563	158	7
CCU	%	16.1	10	8	9	13	23	29	57
Hospital	%	26.6	10	11	14	2	39	48	71
1 Borås	n	214		10	40	73	74	17	–
CCU	%	21.0		–	15	14	28	47	–
Hospital	%	34.1		–	20	27	46	65	–
2. Falun	n	188	–	5	46	57	63	17	–
CCU	%	11.7	–		2	9	19	4	–
Hospital	%	24.5	–	–	13	16	35	53	–
3 Karolinska	n	128		12	27	49	34	6	–
CCU	%	14.8		8	4	14	7	17	–
Hospital	%	26.6		8	11	29	44	17	
4 Linköping	n	89		5	15	30	31	7	1
CCU	%	16.9		20	27	17	13	–	100
Hospital	%	28.1		20	27	27	29	29	100
5 Malmö		181	3	18	39	87	31	3	–
CCU	%	14.9	50	17	18	15	7	33	
Hospital	%	28.7	50	22	23	30	32	67	–
6 Sahlgrenska		199	1	31	74	82	11	–	–
CCU	%	10.1	–	7	4	10	64	–	–
Hospital	%	15.6		10	10	17	64	–	
7 Serafimerlas.		294		18	59	108	87	21	1
CCU	%	12.6			7	9	23	14	–
Hospital	%	23.1	–	11	14	13	43	33	
8. St Göran		104		6	24	36	29	9	–
CCU	%	19.2		–	8	19	24	44	–
Hospital	%	30.8			25	22	45	56	–
9 Uddevalla		260	1	9	26	81	104	37	2
CCU	%	18.8		11	4	16	21	27	100
Hospital	%	28.8		11	4	19	36	51	100
10. Uppsala		136	3	8	17	56	39	12	1
CCU	%	17.6		13	24	13	23	25	–
Hospital	%	25.7		13	24	23	31	42	–
11 Vänersborg		73	1	6	12	24	19	10	1
CCU	%	17.8		17		8	32	40	–
Hospital	%	26.0		17	8	17	42	40	100
12. Östersund		142	1	9	25	46	41	19	1
CCU	%	22.5		11	8	20	29	37	100
Hospital	%	31.7	–	11	8	30	39	58	100

TABLE 3 Previous diseases in relation to age.

Previous disease	Total	Age ≤39	40–49	50–59	60–69	70–79	80–89	≥90
Patients	2008	10	137	404	729	563	158	7
Angina pectoris								
No history	38%	(30%)	50%	42%	35%	3 %	37%	(71%)
<1 month	22	(20)	21	26	23	21	18	–
1–6 months	9	(10)	13	8	10	8	6	(14)
>6 months	26	(10)	9	21	29	31	28	(14)
Uncertain	5	(10)	7	3	3	5	11	(1)
Previous infarction								
None	63	(67)	70	62	60	65	70	(86)
One	26	(33)	24	29	27	4	24	–
Two or more	8	–	5	7	10	8	3	–
Congestive heart failure	24	(10)	7	8	23	35	49	(57)
Hypertension	25	(10)	11	19	28	30	30	(29)
Diabetes	12		6	8	12	15	17	

Congestive heart failure

The frequency of a positive history of congestive heart failure likewise increases with age and there are also highly significant differences between age group except for the 5th and 6th decade

Hypertension and Diabetes mellitus

There is a weak age correlation for hypertension, which increased with age the difference is probably significant between the 5th and 6th decade and highly significant between the 6th and 7th.

For diabetes, too there is a clear age trend but no significant differences between decades.

Delay

The interval between onset of symptoms and admission to the hospital (delay) is related to age in Table 4 There is a tendency for younger patients to be admitted more rapidly than elderly but there are no significant differences between decades. In the interval 1–3 hours the difference between the 6th and 7th decade is probably significant. Comparing patients under 70 with those who were 70 or older the difference is significant for the interval of 1–3 hours and probably significant for the interval of 1–6 and 1–12 hours.

TABLE 4 Time between onset of symptoms and admission to hospital (delay) in relation to age.

Delay in hours	Total	Age ≤39	40–49	50–59	60–69	70–79	80–89	≥90
Patients	2008	10	137	404	729	563	158	7
1–3	30%	(40%)	35%	34%	32%	26%	28%	–
4–6	17	(10)	20	17	17	18	16	(29%)
7–9	8	–	9	8	8	9	6	–
10–1	5	–	5	5	5	5	6	–
13–18	7	(10)	7	6	8	7	6	(14)
19–24	5	–	6	5	5	5	4	(14)
25–48	7	(20)	6	7	8	6	5	(14)
49–98	7	(10)	7	7	8	7	6	(14)
Uncertain	14	(10)	5	11	9	17	23	(14)

Comments

In some earlier reports where the delay has been analysed in more detail, it has been pointed out that older patients came to hospital later than younger (Hacket and Cassem 1969 Moss et al. 1969). This tendency which is supported by the present study is difficult to explain but one reason for it may be that older persons fall ill in a less characteristic picture Uncertain information about the onset of symptoms increased markedly with increasing age which supports this hypothesis. It is also conceivable that more elderly patients have had previous experience of chest pain (angina pectoris) and are therefore prone to wait and see before seeking medical help

Smoking habits

Information about tobacco consumption in relation to age is given in Table 5 There is a strong age-correlation the number of non-smokers increasing continuously with age and the frequence of heavy smokers (more than 20 cigarettes a day) are to be found in younger age-groups.

Comments

Earlier studies have reported that compared with available controls, young male patients with myocardial infarction include very few non-smokers, (Hegglin 1956, Dorken 1967 Hood et al. 1969) The literature on links between smoking and coronary heart disease is vast and contradictory It is agreed, however that the incidence of myocardial infarction in males is related to smoking, especially among young men (Rosenman et al. 1967 Jenkins et al. 1968 Tibblin 1968 and 1972).

The smoking habits in different age groups (Table 5) cannot be used to analyse smoking as a risk factor Smoking habits have changed in recent decades and generally become established in a person s teens. The consumption of cigarettes has increased steadily while that of cigars cigarillos and pipe tobacco has changed very little (information from the National Swedish Social Welfare Board 1968). A further circumstance is the relatively high proportion of smokers in younger age-groups and the large proportion of non-smokers among the elderly In a study of 359 men with AMI discharged alive from hospital Elmfeldt (1974) found a higher tobac consumption in younger patients than in elderly Such age-differences could not be refined in the normal male population in Gothenburg. Glendy et al. (1937) found a striking difference between younger age-groups and elderly with far more total abstainers in elderly than in younger patients. These findings agree well with those in the present study

Symptoms at onset

Some common symptoms at onset are related to age in Table 6.

Chest pain

Chest pain was reported by 85 per cent of the patients and 79 per cent reported such pain with

TABLE 5 Smoking habits in relation to age

Smoking habits	Total	Age ≤39	40–49	50–59	60–69	70–79	80 89	≥90
Patients	2008	10	137	404	729	563	158	7
No smokers	37%	(30%)	12%	17%	33%	52%	67%	(86%)
Ex-smokers	7	–	7	8	7	6	4	–
Pipe-smokers	12	(30)	20	18	13	7	3	–
Smokers, 1 10 cig day	11	–	8	16	13	8	4	–
Smokers, 11–20 cig day	12	(20)	34	21	13	2	–	–
Smokers, 21–50 cig day	2	(20)	11	5	1	–	–	–
Smokers, cigar	2		1	3	2	1	–	–
Uncertain	17	–	7	12	18	24	22	(14)

TABLE 6. Symptoms at onset in relation to age.

Symptoms	Total	Age ≤39	40–49	50–59	60–69	70–79	80–89	≥90
Patients	2008	10	137	404	729	563	158	7
Pain								
No pain	5%	–	2%	3%	4%	8%	11%	–
Oppression	7	–	7	6	9	7	8	(14%)
Pain < 30 min.	6	(10%)	4	6	6	5	5	–
Pain > 30 min.	79	(89)	85	83	78	78	70	(71)
Dyspnoea	42	(33)	31	38	41	44	56	(71)
Dizziness and/or syncope	18	(10)	20	16	20	19	15	(29)
Nausea and/or vomiting	45	(60)	50	46	46	44	39	(57)

a duration of at least 30 minutes. There is a tendency for persistent chest pain (over 30 min.) to be inversely correlated to age. On the other hand chest pain lasting less than 30 minutes seemed to be as common in all old age-groups.

Dyspnoea

A history of shortness of breath and/or rattles in the chest during the current illness was reported 42 per cent. These symptoms increased with and probably reflect left heart failure which ...wise increased with age (see Table 7).

Dizziness and/or syncope

A history of fainting, attacks of dizziness and/or episodes of unconsciousness was reported for 18 per cent of all patients. There was no age correlation.

Nausea and/or vomiting

Nausea and/or vomiting seemed to be very common symptoms at the onset of the current illness, being reported for 45 per cent of the patients. These symptoms were somewhat more frequent at younger ages.

Physical findings on admission and during the first day in CCU

Some clinical findings on admission and during the first day in CCU are related to age in Table 7. One hospital was excluded because data on admission and on the first day were not separated.

Disturbed consciousness

Disturbed consciousness or unconsciousness was noted for 18 per cent of the patients on admission to hospital and for 17 per cent during the first day in CCU. The incidence of these signs shows a clear positive correlation with age.

Left heart failure and frank pulmonary oedema

LHF and frank POe on admission were reported in 21 and 6 per cent respectively. Corresponding figures during the first day were 31 and 8 per cent. Signs of LHF and frank POe increased with age particularly among patients over 70 years of age.

Hypotension and shock

Hypotension on admission was reported for 5 per cent and during the first day for 8 per cent. The corresponding figures for shock were 6 and 11 per cent, respectively. There was no age correlation for hypotension but possibly a weak trend for shock, especially during the first day for which the incidence of shock was significantly higher among patients over 70 years ($p<0.001$).

Comments

Heart failure has been reported in 20 to 70 per cent of patients with acute myocardial infarction (Master et al. 1939, Rosenbaum and Levine 1941, Mintz and Katz 1947, Julian et al. 1964, Lown et al. 1967 a, Restieaux et al. 1967, Marshall et al. 1968, Meltzer 1968, Thomas et al. 1968, Sjögren 1970). Some authors also report that the incidence

TABLE 7 Physical findings on admission and during the first day in CCU in relation to age.

Physical findings	Total	Age						
		<39	40–49	50–59	60–69	70–79	80–89	≥90
Patients	1827	7	119	365	64	532	155	7
Disturbed consciousness								
on admission	18%	–	3%	11%	17%	23%	31%	–
during first day	17	–	7	12	13	23	34	(14%)
Left heart failure								
on admission	21	–	8	15	20	26	35	(29)
during first day	31	–	19	25	30	37	46	(29)
Pulmonary oedema								
on admission	6	–	–	1	6	9	12	
during first day	8		1	3	7	12	16	
Hypotension								
on admission	5		3	4	6	5	7	
during first day	8		7	11	9	10	13	(14)
Shock								
on admission	6		4	4	6	8	8	–
during first day	11		12	7	10	14	16	(29)

One hospital excluded

of congestive heart failure rises progressively with age (Master et al. 1939 Mintz and Katz 1947 Bailey and Beaven 1968, Sjogren 1970) which is in accordance with the present study

The reported incidence of shock due to acute myocardial infarction ranges from 7 to 23 per cent (Braunwald 1967 Killip and Kimball 1967 Lawrie et al. 1967 Lown et al. 1967 a, Wallace et al. 1967 Pentecost and Mayne 1968 Scheidt et al. 1970 Swan et al. 1970 Thompson and Sloman 1971 Wan et al. 1971 Nyquist 1972)

The incidence of shock in the present study was 6 per cent on admission and 11 per cent during the first day The latter figure may seem rather low but refers, as indicated, to the first day of observation not to the whole hospital stay

Arrhythmias

The incidence and age distribution of some important arrhythmias registered on admission are shown in Table 8 and corresponding figure for arrhythmias during the first day in Table 9

For arrhythmias on admission there is a clear age correlation for left bundle branch block, supraventricular tachycardia and atrial fibrillation.

Concerning arrhythmias during the first day (Table 9) two hospitals had to be excluded because they lacked central monitoring equipment for all patients. As with the arrhythmias on admission, there are age-correlations for left bundle branch block and atrial fibrillation. Supraventricular tachycardia, however is now equally common in all age groups. Instead there is an increase with age for A–V block I. Other forms of arrhythmia show no age correlation but it may be worth mentioning that supraventricular bradycardia seemed to be somewhat more common in younger age groups.

Comments

The ECG-recordings on admission have usually been taken for only a short time on the emergency ward, after which the patients have been transported as soon as possible to CCU These recordings on admission cannot be said to give a complete picture of the frequencies of different arrhythmias but they do provide fairly good information and the age distribution agrees with that for the registrations during the first day Some authors report that different arrhythmias increased with age (Johnson and Miner 1958 Hurwitz and Eliot 1964) It has been noted in

TABLE 8 Arrhythmias on admission, in relation to age.

Arrhythmias	Total	<39	40–49	50–59	60–69	70–79	80–89	>90
					Age			
Patients	2008	10	137	404	729	563	158	7
A–V I	3%	–	2%	2%	3%	4%	2%	(14%)
A–V II	1	–	2	1	2	2	–	–
A–V III	2	–	3	1	2	3	3	(14)
LBBB	8	–	1	3	8	12	13	(14)
RBBB	4	–	2	3	3	4	8	(14)
SVT	12	–	6	9	11	15	19	(29)
SVB	4	–	5	4	3	3	2	–
SVEB	6	–	4	6	5	8	5	(29)
A. flutter	1	–	2	2	3	2	1	–
AF	9	–	2	3	6	13	26	–
VEB >5/min.	6	–	5	5	6	9	7	–
VT	2	–	2	2	3	3	1	–

TABLE 9 Arrhythmias during the first day in relation to age.

Arrhythmias	Total	<39	40–49	50–59	60–69	70–79	80–89	>90
					Age			
Patients	1831	9	125	368	669	515	139	6
A–V I	7%	–	3%	3%	6%	10%	9%	(50%)
A–V II	5	–	3	4	5	6	3	–
A–V III	6	–	5	5	5	6	9	(17)
LBBB	9	–	2	5	9	13	14	(33)
RBBB	5	–	5	5	4	5	9	(17)
SVT	24	–	22	24	23	26	24	(50)
SVB	10		13	12	10	10	8	
SVEB	24	(38%)	19	22	24	27	27	(50)
A. flutter	3		2	2	3	4	4	
AF	12	–	2	5	8	20	31	(17)
VEB >5/min	21	–	18	19	21	21	22	(17)
VT	13	–	25	14	13	14	14	(17)
VF	5	(11)	6	4	5	4	7	(17)

Tw hospitals excluded

particular that the frequency of atrial fibrillation is high in old patients (Sloman and Brown 1970 Thompson and Sloman 1971 Helmers et al. (1973) but also that the incidence of bundle branch block increases with age (Thompson and Sloman 1971) Some authors report that patient with heart block tends to be older (Friedberg et al. 1968) and Thompson and Sloman (1971) found a striking increase in the frequency of all forms of bundle branch block but no age correlation for complete A–V block. No age correlation has been found as a rule for ventricular arrhythmias (Sloman and Brown 1970). Dalle and co-workers (1967) did not find any age correlation for ventricular tachycardia but the incidence was high (32 per cent) in the eight decade Lawrie and co-worker (1968) detected no significant age differences in the frequency of ventricular fibrillation though the incidence was higher in patients under fifty

The present study confirms previous findings that left bundle branch block and atrial fibrillation are much more common among older patients. No age correlation was found for ventricular arrhythmias, which also agrees with earlier reports.

ECG-diagnosis on admission and during CCU stay

The site of infarction in relation to age is presented in Table 10. There is no significant age correlation but a tendency towards rather more inferior infarctions in younger age groups. The number of ECG recordings which had been difficult to interpret increased quite clearly in older age groups.

Comments

In an analysis of 359 men with acute myocardial infarction who were discharged alive from hospital, Wilhelmsson (1974) found that inferior infarction was more common in higher ages. On the other hand, in a study of short- and long-term prognosis of 400 patients with acute myocardial infarction, Helmers (1974) found that the mean age of patients with inferior or infero-lateral infarctions was lower than that of all patients discharged from hospital. The later findings are more in accordance with the present results. Studying the influence of the site of myocardial infarction on mortality rates, Isomaki et al (1969) found that electrocardiographic localization was

TABLE 10 Site of infarction on admission and during CCU stay in relation to age

ECG-diagnosis	Total	Age ≤39	40–49	50–59	60–69	70–79	80–89	≥90
Patients	2008	10	137	404	729	563	158	7
No sign of infarction								
on admission	10%	–	10%	8%	10%	11%	17%	–
during CCU-stay	5		4	3	4	6	11	–
Anterior								
on admission	25	(50 %)	21	27	27	22	20	(43 %)
during CCU-stay	32	(60)	29	37	35	27	27	(57)
Lateral								
on admission	2		4	2	3	3	2	
during CCU-stay	4		8	5	5	4	3	–
Inferior								
on admission	19	(20)	26	20	20	18	11	
during CCU-stay	25	(20)	33	27	24	23	18	–
Antero-lateral								
on admission	2	(10)	4	2	2	2	1	–
during CCU-stay	4	(10)	7	4	3	5	3	–
Infero-lateral								
on admission	2		2	4	2	1	2	–
during CCU-stay	4		5	7	3	2	2	–
Combined								
on admission	1		1		1	1	1	–
during CCU-stay	2	–	1	1	2	2	2	–
Uncertain								
on admission	39	(20)	32	37	35	42	46	(57)
during CCU-stay	24	(10)	13	16	24	31	34	(43)

Indicates ECG-changes over anterior-inferior as well as over anterior-inferior and lateral walls.

impossible in about 11 per cent and that these patients were older and had a higher frequency of hypertension, left bundle branch block, previous infarction and shock than the others. This is well in line with the present finding that older patients had a higher frequency of other ECG-changes as bundle branch block and atrial fibrillation, making the interpretation of ECG more difficult and possibly explaining the high figures for indefinite ECG in these age groups.

Maximum SGOT

Max. SGOT-values in relation to age are presented in Table 11 These values show no age correlation, though large myocardial infarctions (<200 units ml) seem to be more common in younger age groups.

Comments

Elevated serum transaminase activity especially SGOT when recorded daily and started less than 24 hours after the suspected infarction, indicates destruction of tissues, presumably heart muscle d correlates strongly with acute myocardial farction (Blórck and Hansson 1956 Chinsky et al. 1956, Sampson 1958 Wroblewski 1959 Agress and Kim 1960, West et al. 1966 Kibo and Nilsson 1967). In Wilhemsson s (1974) study of 359 men with AMI discharged alive from hospital, there was no age trend for SGOT-level. No other reports have been found concerning age and SGOT level in acute myocardial infarction.

The proportion (8 per cent) with no rise in SGOT-values seems rather high but part of the reason may be that many patients died early probably before a maximum had been reached. Furthermore some patients entered hospital rat her late so that a maximum may have been passed before their admission. No SGOT data were available for 5 per cent of the material, mainly among high age groups. This is partly explained by the fact that many of these patients had been admitted in poor condition and many of them had died early so that no blood samples were available for enzyme determination.

Drug therapy during the first day

About every third patient has been treated with diuretics (37 per cent) some kind of cardiac glycoside (34 per cent) or lidocain infusion (33 per cent). Atropin has been given to 18 per cent of the patients.

Comments

Many old patients were treated with diuretics and digitalis and this is surely explained by the higher incidence of LHF in elderly Almost every second patient over 70 years of age has been treated with one or both these drugs, the difference compared with patients under 70 being significant. Treatment with lidocain is much the same in all age groups, which correspond to the lack of age correlation for ventricular arrhythmias. Treatment with atropin seems to be somewhat more common among younger age groups, as a consequence of the fact that bradyarrhythmias were more common in these age groups.

TABLE 11 Maximum SGOT in relation to age

SGOT units per ml	Total	Age ≤39	40–49	50–59	60–69	70–79	80–89	≥90
Patients	2008	10	137	404	729	563	158	7
1–39	8%		8%	9%	7%	7%	10%	(29%)
40–59	10	(10%)	10		10	12	10	–
60 99	18	(10)	18	17	18	20	15	–
100–149	17	(20)	14	20	18	14	18	(29)
150–199	12	–	12	13	13	9	13	–
200–299	15	(20)	18	17	15	14	13	(29)
≥300	15	(40)	19	16	14	15	10	(13)
No data	5	–	1	1	5	9	11	–

Sex, like age is held to be a very important variable in ischaemic heart disease Males and females have been considered separately in many studies. In the present study this is done only in the present section, in which males and females have been analysed for the same variables as the age factor in the preceding section.

Mortality and sex

Of the total material of 2008 patients 1447 were men (72.1 per cent) and 561 women (27 9 per cent), giving a male/female quotient of 2.6 The age related mortality in CCU and while in hospital is shown for men and women in Table 12 The mortality in CCU was 14.3 per cent for men and 20 7 per cent for women and the total mortality was 23 8 and 34.0 per cent, respectively The total mortality was significantly higher for women, both in CCU and while in hospital ($p<0.01$ and $p<0.001$), but there were no significant sex differences within the various decades.

Comments

Men dominate in nearly all series of acute MI (Master et al. 1939 Rosenbaum and Levine 1941 Billings et al. 1949 Honey and Truelove 1957 Harnagel et al. 1959 Hughes et al. 1963 Killip and Kimball 1967 Lawrie et al. 1967 Eddy and Mackinnon 1970) especially in younger age groups (*Mintz and Katz 1947 Doscher and Poindexter* 1950 Smith and Denham 1951 Harnagel et al. 1959 Wahlberg 1963 Bailey and Beaven 1968, Linko et al. 1970). The present male/female quotient of 2.6 is well in line with other reports, where it usually lies in the interval *2.0–3.5* (Master et al. 1939 Mintz and Katz 1947 Billings et al. 1949 Harnagel et al. 1959 Hughes et al. 1963 Wahlberg 1963 Julian et al. 1964 Killip and Kimball 1967 Lawrie et al. 1967 Bailey and Beaven 1968) Values below 2.0 have been reported in some studies (Helander 1949 Lindén 1952, Biorck et al 1957 Hofvendahl 1971) and values above 4 0 in others (Doscher and Poindexter 1950 Smith and Denham 1951 Hughes et al. 1963 Restieaux et al. 1967 Thomas et al 1968).

Most studies have not shown any significant differences in mortality between the sexes (Master et al 1939 Rosenbaum and Levine 1941 Helander 1949 Harnagel et al. 1959 Wahlberg 1963 Julian et al. 1964 Lawrie et al 1967 Linko 1970 Helmers 1974) A poorer prognosis for women has been found in some studies (Chambers 1946 Mintz and Katz 1947 Doscher and Poindexter 1950 Zinn and Cosby 1950 Bailey and Beaven 1968 Bovegård et al. 1970 Nielsen 1970) and is probably explained in part by a higher mean age In addition to this, Rosenbaum and Levine (1941) pointed out that a larger proportion of women have a history of hypertension. Thompson and Sloman (1971) found a strikingly high mortality among females under 60 compared with men, 30.0 and 7 3 per cent, respectively In the present study *the significantly higher total mortality for women* is explained by their higher mean age because no significant differences were found within the

TABLE 12. Mortality by age, in CCU and in hospital among males and females.

Sex	Total	≤39	40–49	50–59	60–69	70–79	80–89	≥90
Males	1447	9	126	349	526	351	83	3
CCU	14 3%	13%	6%	9%	13%	22%	28%	67%
Hospital	23 8	13	10	15	21	37	50	67
Females	561	1	11	55	203	212	75	4
CCU	20 7%	–	27%	6%	15%	26%	29%	50%
Hospital	34 0	–	27	15	25	43	47	75

various decades. The high mortality (27 per cent) for women in the 4th decade is not significantly different from that for the corresponding age group of men and there are too few women in this decade to warrant any conclusions about the high figure.

Previous diseases

Previous diseases among males and females are listed in Table 13. Concerning an earlier history of chronic angina pectoris and previous myocardial infarction, the differences between the two sexes were only probably significant ($p<0.05$). The incidence of LHF, hypertension and diabetes were much higher among females, there differences being highly significant.

TABLE 13. Previous diseases among males and females.

Previous diseases	Males	Females	P
Patients	1447	561	
Angina pectoris			
None	39%	36%	N.S.
<1 month	23	20	N.S.
1–6 months	10	8	N.S.
>6 months	25	29	<0.05
Uncertain	3	7	<0.05
Previous infarction			
None	63	65	N.S.
One	28	23	<0.05
Two or more	8	9	N.S.
Congestive heart failure	20	37	<0.001
Hypertension	19	43	<0.001
Diabetes	10	18	<0.001

Comments

The prevalence of angina pectoris has been found to be about the same among males and females in many studies (Rosenbaum and Levine 1941, Doscher and Poindexter 1950, Smith and Denham 1951, Honey and Truelove 1957, Wahlberg 1963). In the Framingham Study (Gordon and Kannel 1971) the milder forms of CHD were much more common among women than men. AP accounted for two thirds of the cases in women and for little more than one third in men. In a study of ischaemic heart disease in women, Bengtsson (1973) found that the prevalence of MI was much higher in men than in women, while that of AP was about the same in both sexes at the ages at 50 and 54 years. In a community study from Edinburgh concerning 1858 episodes of suspected heart attacks in the population under the age of 70, Armstrong et al (1972) found that women had a lower proportion of myocardial infarction and a higher proportion of ischaemia.

Hypertension is reported to be much more common among women than men (Master et al. 1939, Fisher and Zukerman 1946, Mintz and Katz 1947, Billings et al. 1949, Korhonen and Koskinen 1960, Bailey and Beaven 1968) and diabetes is also more frequent among women (Billings et al. 1949, Doscher and Poindexter 1950, Zinn and Cosby 1950, Smith and Denham 1951, Bailey and Beaven 1968).

In the present study the somewhat higher prevalence of chronic AP among women could perhaps partly be explained by the age factor as chronic AP increases with age (Table 3).

As males dominate in most materials of patients with AMI, one would expect men to show a higher prevalence of previous MI, but the present difference between the sexes was only probably significant.

The higher prevalence of CHF, hypertension and diabetes among women cannot be explained by the age factor alone, because this significance remains in some age groups even when a comparison between males and females was done in the same decade, especially for CHF and hypertension.

Delay

Thirty-two per cent of the males and twenty-six per cent of the females arrived at hospital within three hours and this difference is significant. In other time intervals there were no significant differences.

Comments

Hacket and Cassem (1969) found no significant differences among the two sexes. On the contrary Moss et al. (1969) found that women had a considerably greater decision time than men but because of large intra-group variations the difference between the sexes was only probably signi-

ficant. This agrees well with the findings in the present study

The longer delay-time for women in the present study can possibly partly be explained by such factors as higher mean-age and the higher prevalence of chronic angina pectoris among women.

Smoking habits

The frequency of smokers was higher among males, as can be seen in Table 14. The differences between the sexes were highly significant except for moderate smokers (1–10 cigarettes daily) for whom the difference was only probably significant. However among younger age-groups the differences between males and females were not significant.

TABLE 14 Smoking habits among males and females

Smoking habits	Males	Females	P
Patients	1447	561	
Non smokers	23%	71%	<0.001
Ex-smokers	9	3	<0.001
Pipe-smokers	17	–	<0.001
Smokers 1–10 cig. a day	12	8	<0.05
Smokers 11–20 cig. a day	15	6	<0.001
Smokers >20 cig. a day	3	1	N.S
Smokers cigar	2	1	N.S

Comments

Smoking was found to be more common in younger patients than in older age-groups, both among men (Elmfeldt 1974) and women (Ellertsen and Sulheim 1970, Bengtsson 1973) and the same applies in the present study

Symptoms at onset and physical findings during the first day in CCU

There were no significant differences between males and females concerning symptoms at onset and physical findings during the first day in CCU except for dyspnoea and left heart failure with much higher prevalences for women, the differences being highly significant. However these sex differences disappeared when the comparison was made between males and females within the same decade

Arrhythmias during the first day in CCU

There were no significant differences between males and females concerning most arrhythmias, exceptions being LBBB SVT and AF for which the incidences were higher among women. The difference was probably significant for LBBB and significant for SVT and AF

Comments

The higher incidence of LBBB and AF among females is perhaps partly explained by the age factor because the incidence of these arrhythmias increases with age. By analogy the higher incidence of SVT among women can be explained by the higher frequency of LHF among females because SVT is often a sign of LHF

ECG-diagnosis and Maximum SGOT

No significant differences were found between males and females concerning the site of infarction and maximum SGOT. An exception was anterior infarction, for which the frequency was higher for men (34 per cent) than for women (27 per cent), the difference being significant.

A previous history of angina pectoris earlier myocardial infarction, congestive heart failure hypertension and diabetes mellitus have been analysed in relation to most of the other data. The prevalence rates and mortality figures are presented first, then the relationship to other previous diseases, clinical findings site of infarction, arrhythmias and max. SGOT

Prevalence rates

Angina pectoris

758 patients (38 per cent) reported no history of angina pectoris 445 patients (22 per cent) chest pain for less than one month, 184 patients (9 per cent) symptoms for 1 -6 months and 521 patients (26 per cent) for more than six months (Table 3) Of the 445 patients who reported chest pain for less than one month, 70 per cent had had pain for 1–3 days, 10 per cent for 4–7 days and 20 per for 2–4 weeks prior to admission.

...ious myocardial infarction

1273 patients (63 per cent) had not had previous myocardial infarction 527 (26 per cent) had had one and 180 (9 per cent) two or more infarctions earlier

Previous congestive heart failure hypertension and diabetes mellitus

487 patients (24 per cent) mentioned symptoms of previous CHF 509 patients (25 per cent) had hypertension and 240 (12 per cent) were diabetics.

Mortality

The mortality grouped according to the patients previous diseases, is presented in Table 15

Angina pectoris

Patients with chest pain for more than six months had a higher hospital mortality than the other groups. The difference in mortality is probably significant during the CCU-stay and significant for the total hospital stay

Previous myocardial infarction

Patients with previous MI had a higher hospital mortality than those with a first infarction, the difference being probably significant.

Congestive heart failure, hypertension and diabetes

Patients with a history of CHF had a higher mortality than the rest of the material. The differences are highly significant both during the CCU-stay and in after-care In contrast patients with hypertension had no increased mortality

Diabetic patients had a higher mortality than the rest of the material. The difference in mortality is probably significant during the CCU-stay significant during after-care and highly significant for the total stay in hospital.

TABLE 15 Mortality among patient with previous angina pectoris, myocardial infarction, congestive heart failure, hypertension and diabetes mellitus

Mortality	Total	Angina pectoris				Previous infarction			CHF	Hypertension	Diabetes
		None	< 1 m	1–6 m	> 6 m	None	One	Two or more			
Patient	2008	758	445	184	521	1273	527	180	487	509	240
CCU	16 1%	15%	13%	16%	19%	15%	16%	22%	21%	15%	21%
After-care	10 5	10	10	9	12	10	13	8	16	12	15
Hospital	26 6	25	23	25	31	25	29	30	37	27	36

Comments

Angina pectoris

Previous AP has been mentioned as a favourable factor by some authors in that it stimulates a collateral circulation (Rosenbaum and Levine 1941). Many authors have found no correlation between previous AP and the short term results for pateints with acute MI (Mintz and Katz 1947 Billings et al. 1949 Smith and Denham 1951 Honey and Truelove 1957 Harnagel et al. 1959 Peel et al. 1962, Hughes et al. 1963 Wahlberg 1963 McGuire and Kroll 1972) Others have found that previous AP appears to have an unfavourable though minor influence (Doscher and Poindexter 1950 Beard et al. 1960 Norris et al. 1968)

In this series patients with a history of chronic AP lasting more than six months had a higher mortality but the difference probably depends on the age factor because a larger proportion of older patients had a history of chronic AP (Table 4). Hughes and co-workers (1963) found that AP is of no great importance for the short-term result but that in conjunction with other complications it may be somewhat unfavourable for the prognosis.

Previous myocardial infarction

A higher mortality among patients with previous MI compared with those with a first infarction has been reported in several investigations (Master et al. 1939 Fisher and Zukerman 1946 Doscher and Poindexter 1950 Smith and Denham 1951 Harnagel et al. 1959 Hughes et al 1963 Lemlich 1965 Helmers 1974). Isacsson and co-workers (1969) reported that serious complications (VF asystole progressive myocardial insufficiency) increased with the number of previous MI with a marked difference in mortality Other studies yielded no significant differences in mortality between patients with a first infarction and those with re-infarction (Lindén 1952, Honey and Truelove 1957 Peel et al. 1962 Norris et al 1968 Ballock et al. 1970)

In the present study patients who had suffered previous MI had a somewhat higher hospital mortality but the difference is only probably significant There are no significant differences between the groups with chronic AP one infarction or two or more infarctions earlier Similar results have been reported by Norris and co-workers (1969 a)

Congestive heart failure

A previous history of CHF has been regarded as an unfavourable prognostic factor by several authors (Chambers 1946 Billings et al. 1949 Lindén 1952, Honey and Truelove 1957 Harnagel et al. 1959 Bailey and Beaven 1968 Thompson and Sloman 1971 Mc Guire and Kroll 1972, Little is actually known about its prognostic importance, probably because patients with CHF are a heterogeneous group and their symptoms can be due to other diseases, e g. valvular heart disease hypertension, arrhythmias, chronic obstructive pulmonary disease etc. A history of effort dyspnoea of uncertain etiology is reportedly associated with an unfavourable prognosis (Peel et al. 1962)

In the present study a history of CHF is associated with an increased mortality but allowance should be made for the age factor

Hypertension

Patients with AMI and a history of hypertension have an increased mortality according to some authors (Rosenbaum and Levine 1941 Doscher and Poindexter 1950 Harnagel et al. 1959 Beard et al 1960) but not according to others (Fisher and Zukerman 1946, Mintz and Katz 1947 Billings et al. 1949 Smith and Denham 1951 Lindén 1952, Honey and Truelove 1957 Korhonen and Koskinen 1960 Hughes et al. 1963 Wahlberg 1963 Bailey and Beaven 1968 Norris et al 1968 Thompson and Sloman 1971 Helmers 1974). In this material the mortality among patients with hypertension was no higher than among those without. Hughes et al. (1963) mentioned that hypertension alone does not tend to increase mortality but may do so when combined with other complications.

Diabetes mellitus

Although some studies show that patients with diabetes do not have an increased mortality in AMI (Doscher and Poindexter 1950 Smith and

Denham 1951 Harnagel et al 1959 Korhonen and Koskinen 1960 Wahlberg 1963 Norris et al 1968 Thompson and Sloman 1971 Helmers 1974) many authors have found that diabetes has an unfavourable influence on the short term prog nosis (Master et al 1939 Mintz and Katz 1947 Billings et al 1949 Zinn and Cosby 1950 Lindén 1952 Honey and Truelove 1957 White et al 1960 Hughes et al, 1963 Bally and Beaven 1968 McGuire and Kroll 1972)

In the present study patients with diabetes mellitus had a markedly increased hospital mor tality but here too the age factor must be taken into consideration as diabetes is age-correlated (Table 4)

Previous diseases

The interrelationship of different previous diseases is presented in Table 16

Angina pectoris

Of 521 patients with chronic AP (more than six months), 49 per cent had suffered previous MI hich is significantly more than the 22 per cent of tients without AP ($p<0.001$). Highly significant differences were also found between patients with chronic AP and those without angina for earlier CHF (44 against 14 per cent) and for hypertension (34 against 19 per cent). For diabetes mellitus there was a similar tendency (15 against 11 per cent) but this is only probably significant ($p<0.05$)

Previous myocardial infarction

1273 patients had not had MI before Of these, 20 per cent had chronic AP The corresponding figure for patients with one previous infarction earlier was 33 per cent and for patients with two or more infarctions 44 per cent. The difference between the two first groups is highly significant and between the latter significant.

Previous symptoms of CHF were mentioned by 19 per cent of the patients with no earlier MI by 27 per cent of those with one infarction before and by 51 per cent of those with two or more before. The differences between these groups are highly significant.

No differences between the groups were found in the prevalence of either hypertension or diabetes mellitus.

Congestive heart failure

487 patients (24 per cent) had a history of CHF Of these 47 per cent had chronic AP which differs highly significantly from the incidence in the rest of the material. Highly significant differences

TABLE 16. Interrelationship of previous diseases

Previous diseases	Total	Angina pectoris				Prev infarction			CHF	Hyper tension	Diabetes
		None	< 1 m	1–6 m	> 6 m	None	One	Two or more			
Patients	2008	758	445	184	521	1273	527	180	487	509	240
Angina pectoris											
Total	57%					49%	67%	82%	73%	68%	61%
< 1 month	22					20	25	28	17	25	22
1–6 months	9					9	9	10	9	8	7
> 6 months	26					20	33	44	47	35	32
Prev infarction											
Total	34	22%	40%	37%	49%				46	31	39
One	26	20	30	27	34				29	25	28
Two or more	8	2	10	10	15				17	6	11
CHF	24	14	19	23	44	19	27	51		36	41
Hypertension	25	19	28	23	34	27	24	21	37		33
Diabetes	12	11	12	9	15	11	13	17	20	16	

were also registered for this patient group concerning the prevalence of previous MI hypertension and diabetes compared with the rest of the material.

Hypertension

509 patients (25 per cent) had hypertension. Of these, 35 per cent had chronic AP The difference from the rest of the material is highly significant

The prevalence of previous MI was the same as for the whole material.

Symptoms of CHF were much more common among patients with hypertension the difference compared with the rest of the material being highly significant. Patients with hypertension also had a significantly higher prevalence of diabetes.

Diabetes mellitus

240 patients (12 per cent) had diabetes and 41 per cent of them had a history of CHF which is significantly higher ($p<0.001$) than in the rest of the material. Hypertension was also significantly more common than among the patients without diabetes but this was not so for either earlier MI or AP

Physical findings

Physical findings – LHF POe hypotension and shock on admission and during the first day – are related to previous diseases in Table 17 One hospital has been excluded from the calculations concerning the first day (see page 22).

Angina pectoris

Patients with AP for less than six months did not differ from those who denied earlier AP concerning the physical findings listed in Table 17 The incidences of LHF and POe during the first day were significantly higher for patients with chronic AP than among the rest of the patients. The incidences of hypotension and shock were almost the same in the different groups.

Previous myocardial infarction

The incidences of physical findings did not differ significantly between patients without previous MI and those with one infarction earlier Patients with two or more infarctions earlier had significantly higher incidences of LHF and shock on admission than the rest of the patients. During the first day those with several MI displayed a higher incidence of POe ($p<0.01$).

TABLE 17 Physical findings on admission and during the first day in relation to previous diseases.

Physical findings	Total	Angina pectoris: None	Angina pectoris: <1 m	Angina pectoris: 1–6 m	Angina pectoris: >6 m	Prev infarction: None	Prev infarction: One	Prev infarction: Two or more	CHF	Hypertension	Diabetes
Patients	1827	682	411	161	483	1168	460	172	460	477	229
On admission											
LHF	21%	18%	16%	20%	27%	20%	21%	27%	35%	22%	28%
Pulm. oedema	6	5	6	2	7	5	6	9	14	7	9
Hypotension	5	4	5	4	5	5	5	6	5	5	5
Shock	6	7	3	5	6	5	6	11	7	4	8
During first day											
LHF	31%	30%	25%	29%	37%	30%	34%	31%	46%	33%	37%
Pulm. oedema	5	3	3	1	10	4	5	11	12	6	7
Hypotension	8	9	6	8	9	8	9	7	9	7	7
Shock	9	9	4	8	11	8	9	14	12	7	8

One hospital excluded

Congestive heart failure

Patients with a history of CHF had a higher incidence of LHF both on admission and during the first day than the rest of the material. This difference is highly significant.

Hypertension and diabetes mellitus

Patients with hypertension did not differ from the rest of the material concerning physical findings on admission and during the first day. Patients with diabetes had a significantly higher incidence of LHF both on admission and during the first day than patients without diabetes.

Arrhythmias during the first day

Arrhythmias during the first day are related to previous diseases in Table 18. Two hospitals have been excluded because they lacked the facilities for continuous monitoring of all patients during the whole CCU-stage (see page 23).

... pectoris

... with chronic AP had higher incidences of ... 3 and SVT than the rest of the material. The differences were highly significant. These patients had also a greater tendency to develop VT and VF than other patients ($p<0.01$). Otherwise there were no or only small differences in the occurrence of different arrhythmias compared with the rest of the material.

Myocardial infarction

Patients with one myocardial infarction earlier had somewhat higher incidences of A V block II, A-V block III, LBBB and VT but the differences are only probably significant.

Patients with two or more infarction earlier had a higher incidence of LBBB than the rest of the material and this difference is highly significant. These patients also had a higher tendency to develop VT and VF during the first day ($p<0.05$).

Congestive heart failure

Patients with a history of CHF had higher incidences of several arrhythmias compared with the other patients. These differences are highly significant for A V block I, LBBB, AF, frequent VEB and VT.

Hypertension and diabetes mellitus

Patients with earlier history of hypertension had a higher incidence of SVT than those without hypertension, the difference being highly significant.

TABLE 18. Arrhythmias during the first day in CCU in relation to previous diseases.

		Angina pectoris				Prev. infarction					
Arrhythmias	Total	None	<1 m	1–6 m	>6 m	None	One	Two or more	CHF	Hyper-tension	Diabetes
Patients	1831	693	398	165	484	1173	479	160	444	450	211
A–V block I	7%	6%	7%	6%	9%	7%	7%	6%	12%	6%	10%
A–V block II	5	5	4	1	6	4	4	7	5	5	5
A–V block III	6	7	4	6	5	7	3	8	6	4	8
LBBB	9	7	7	9	14	8	11	19	20	11	16
RBBB	5	5	6	4	4	6	4	4	6	4	3
SVT	24	21	22	16	30	22	26	31	29	30	29
SVB	10	12	9	9	10	11	8	11	9	10	4
AF	12	11	11	10	16	13	10	14	26	14	19
VEB>5 min	21	21	16	23	24	21	17	25	28	25	22
VT	13	14	10	11	17	13	12	19	19	13	11
VF	5	4	3	4	7	4	7	9	5	4	4
Asystole	10	11	9	9	11	10	11	14	12	10	11

Two hospitals excluded

Otherwise patients with and without earlier hypertension did not differ concerning the occurrence of different arrhythmias.

Patients with diabetes showed significantly higher incidences of LBBB and AF than other patients. The incidence of SVB was lower for patients with diabetes than for the rest of the material ($p<0.01$).

Maximum SGOT

High SGOT-values (<200 units/ml) were significantly less frequent among patients with re-infarctions (7 per cent) than among those with a first infarct (18 per cent). No other obvious association was found between maximum SGOT and previous diseases.

The delay (the interval between onset of symptoms and arrival at hospital) has been analysed against other available factors in the present study The correlation between age and delay has already been discussed (page 20) Any correlations between delay and other factors are discussed in the sections on these. This section is devoted to the *distribution and mortality for intervals of different* duration.

Frequency distribution

Table 4 shows the interval between onset of symptoms and admission to hospital. 30 per cent of the patients came to hospital within 3 hours, 47 and 60 per cent, respectively within 6 and 12 hours, 72 per cent within 24 hours and 79 per cent less than 48 hours after the onset of symptoms.

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19 gives the mortality as a percentage of the number of patients who were admitted within different intervals. The mortality was highest in the group with an uncertain onset of symptoms, the difference is significant compared with the rest of the material.

Comments

The delay for patients with AMI varies greatly in different investigations (Beard et al. 1960· Lawrie et al. 1967 Lown et al. 1967 a, Baily and Beaven 1968 Norris et al. 1968 Pentecost and Mayne 1968 Woodhouse and Hunter 1968 Hackett and Cassem 1969 Isacsson et al. 1969) and the variations may help to explain some of the differences in hospital mortality A majority of deaths in AMI occur outside hospital. In several investigations it has been found that more than 40 per cent of the deaths in MI occurred within one hour after the onset of symptoms, the incidence among young and middle-aged men being as high as 50–60 per cent (Yater et al. 1948 Spiekerman et al. 1962, *Bainton and Peterson 1963* Paintridge and Geddes 1967 Adgey et al. 1968 MacNeilly and Pemberton 1968 Fulton et al. 1969 Kuller 1969 Moss et al. 1969 Paintridge 1970 Adgey et al. 1971 Gordon and Kannel 1971 Armstrong et al. 1972). In Swedish studies about 35 per cent of the deats in IHD occurred outside hospital (Fodor 1969· Wikland 1971) and in about 50 per cent of the medically unattended fatal cases with known duration of symptoms, which could be related to the last attack of cardiac symptoms, death occurred within 15 minutes of the onset of these symptoms (Wikland 1971)

The cause of delay has been analysed in detail by some authors (Hackett and Cassem 1969 Moss et al. 1969 Armstrong et al. 1972) and the importance of delay for mortality has been discussed at length (Lawrie et al. 1967 Lown et al. 1967 a, Norris et al. 1968 Woodhouse and Hunter 1968) because wide variations in the interval between onset of symptoms and admission to hospital can greatly influence hospital mortality especially during the early phase A high incidence of arrhythmias within the first hour after the debut of symptoms has been reported from investigations with mobile CCU (Paintridge and Geddes 1967 Adgey et al. 1971). Most of the deaths in the early phase of AMI tend to be due to arrhythmias (Mower et al. 1964 Lown et al.

TABLE 19 Mortality in relation to delay

		Delay in hours							
Mortality	Total	1–3	4–6	7–9	10–12	13–24	25–48	49–98	Uncertain
Patients	2008	610	347	160	100	233	140	142	212
CCU	16.1%	17%	15%	16%	18%	10%	9%	16%	26%
After-care	10.5	8	9	13	7	12	11	11	16
Hospital	26.6	25	24	28	25	23	21	27	42

1967 a) and VF is probably the cause of death in the majority of these cases (Paintridge and Geddes 1967 Lown et al. 1969 Adgey et al. 1971).

Some authors found no significant differences in mortality in relation to different intervals (Beard et al. 1960 Norris et al. 1969 a) which is in accordance with the present study Patients with a short delay are reported to have a better prognosis by some authors (Billings et al 1949) but a worse prognosis by others (Linko 1954 Lawrie et al 1967 Pentecost and Mayne 1968)

The high mortality for the present group, with an uncertain onset of symptoms is probably explained by the fact that many of these patients were in a very poor condition on arrival at hospital. Old patients were also overrepresented in this group (Table 4).

In this section signs of power failure (LHF and POe *hypotension and shock) both on admission* and during the first day have been analysed in relation to most available variables but one hospital has been excluded, see page 22 (including Tables 20—23) The prevalence rates and mortality figures are given first, followed by the relation to delay symptoms at onset previous diseases and arrhythmias.

Prevalence rates

LHF was seen in 21 per cent *and* pulmonary oedema in 6 per cent on admission to hospital, and in 31 and 8 per cent, respectively during the first day in CCU (Table 7).

Hypotension and shock were reported in 5 and 8 per cent, respectively on admission, the corresponding figures during the first day in CCU were 6 d 11 per cent.

/

ie mortality among patients with signs of LHF and POe on admission to hospital and during the first day is shown in Table 20 These categories of patients had a higher mortality than the rest of the material both during the stay in CCU and during after-care, and thus for the total stay in hospital ($p<0.001$).

The mortality among patients with hypotension and shock on admission to hospital and during the first day in CCU is shown in Table 20 Patients with shock had, as could be expected, a very high mortality during the stay in CCU with highly significant differences compared to patients without shock. *Hypotension* was also associated with a high mortality the difference compared to patients without hypotension being highly significant for the total hospital stay

Comments

Many authors have pointed out that the prognosis in AMI is much worse for patients with signs of LHF than for those without these symptoms (Rosenbaum and Levine 1941 Mintz and Katz 1947 Smith and Denham 1951 Honey and Truelove 1957 Harnagel et al. 1959 Beard et al. 1960; Peel et al. 1962, Hughes et al. 1963 Wahlberg 1963 Lemlich 1965 Bailey and Beaven 1968 Meltzer 1968 Norris et al. 1968 Hofvendahl 1971 Sjögren 1970; Thompson and Sloman 1971 Helmers 1974).

Direct comparisons between mortality figures in different investigations are meaningless owing to variations in the definition of subjects with LHF

The age distribution can greatly influence the mortality rate and consequently the evaluation of heart failure as a prognostic factor White and co-workers (1960) found that heart failure was seen most frequently in older patients and significantly increased the fatality rate only in these.

The high mortality figures for the present patients with LHF and POe are in accordance with many other studies. The high age correlation for LHF in this material (Table 7) may be partly responsible for the high mortality figures for patients with these symptoms.

TABLE 20. Mortality in relation to physical findings on admission and during the first day

		On admission				During first day			
Mortality	Total	LHF	POe	Hypo-tension	Shock	LHF	POe	Hypo-tension	Shock
Patients	1827	382	104	91	116	578	146	176	202
CCU	16 2%	29%	26%	36%	64%	25%	31%	23%	69%
After-care	10 2	17	18	13	14	15	20	16	12
Hospital	26 4	46	44	49	78	40	51	39	81

One hospital excluded.

The high mortality for patients with LHF and POe during after-care agrees with the findings of Honey and Truelove (1957). Sjogren (1970) found a high CCU-mortality in heart failure for patients previously compensated, whereas failure in patients with a past history of decompensation was associated with an increased mortality during after-care.

Hypotension and shock have been cited as very unfavourable prognostic signs in AMI (Rosenbaum and Levine 1941 Mintz and Katz 1947 Billings et al. 1949 Helander 1949 Wållgren 1950 Linko 1954 Binder et al. 1955 Honey and Truelove 1957 Biörck et al. 1957 Beard et al. 1960 Malach and Rosenberg 1960· White et al. 1960 Peel et al. 1962, Hughes et al. 1963 Wahlberg 1963 Day 1965 Lemlich 1965 Goble et al. 1966 Braunwald 1967 Killip and Kimball 1967 Lawrie et al. 1967 Lown et al. 1967 b Wallace et al. 1967 Shubin and Weil 1967 Norris et al. 1969 a, Bloomfield et al. 1970· Scheidt et al. 1970 Hofvendal 1971 Thompson and Sloman 1971 Wan et al 1971 Nyquist 1972 Helmers 1974).

As in the case of LHF the differences in patient groups and in definitions of hypotension and shock make it extremely difficult to compare different series. As a rule the mortality is high in all investigations and the present figures for patients with hypotension and shock are in accordance with many other studies (Rosenbaum and Levine 1941 Peel et al 1962, Wahlberg 1963 Day 1965 Goble et al. 1966 Braunwald 1967 Killip and Kimball 1967 Lawrie et al. 1967 Lown et al. 1967 b Wallace et al. 1967 Bloomfield et al. 1970 Scheidt et al. 1970· Swan et al. 1970 Wan et al. 1971 Nyquist 1972)

The establishment of CCUs has not been able to decrease the mortality for patients with severe complications such as POe and shock (Killip and Kimball 1967 Lawrie et al. 1967 Meltzer 1968 Sloman et al. 1968 Norris et al. 1969 b Bloomfield et al. 1970· Scheidt et al. 1970· Sloman and Brown 1970; Hofvendahl 1971). Trials with assistant circulation for patients in shock have not been particularly successful (Kantrowitz et al. 1969· Soroff et al. 1969 Nyquist 1972).

Previous diseases

Table 21 relates previous diseases to physical findings on admission and during the first day

A history of chronic AP was noted for 34 per cent of the patients with LHF on admission and for 31 per cent of those with LHF during the first day These rates are significantly higher than in the rest of the material.

There was a history of previous CHF for 42 per cent of the patients with LHF on admission and for 35 of those with LHF during the first day The

TABLE 21 Previous diseases in relation to physical findings on admission and during the first day

Previous diseases	Total	On admission: LHF	On admission: POe	On admission: Hypotension	On admission: Shock	During first day: LHF	During first day: POe	During first day: Hypotension	During first day: Shock
Patients	1827	382	104	91	116	578	146	176	202
Angina pectoris									
None	37%	33%	32%	33%	43%	35%	27%	39%	40%
< 1 month	23	18	22	23	10	20	20	17	12
1–6 months	9	8	3	7	7	8	3	10	7
> 6 months	26	34	35	29	25	31	44	28	31
Infarction									
None	64	62	53	65	55	62	51	66	57
One	25	25	28	23	25	26	27	23	27
Two or more	8	12	13	10	10	10	17	9	11
CHF	25	42	61	28	28	35	58	28	32
Hypertension	26	28	33	15	17	27	32	19	21
Diabetes	13	17	19	12	16	15	18	9	12

One hospital excluded

differences from the rest of the material are highly significant.

The prevalences of previous MI hypertension and diabetes did not differ significantly from other patients.

Forty-four per cent of the patients with POe during the first day had a history of chronic AP which is highly significantly more than in the rest of the material, and 17 per cent had had two or more MI earlier the difference again being significant. Furthermore 61 per cent of the patients with POe on admission and 58 per cent of those with POe during the first day had a history of CHF these rates likewise being highly significantly different from those among patients with no signs of LHF

Patients with hypotension did not differ in any respect from the rest of the material concerning previous diseases.

Thirty-one per cent of the patients with shock during the first day had a history of chronic AP and 32 per cent had a history of previous CHF these figures are significantly higher than those for other patients.

/he delay in relation to physical findings on admission and during the first day is shown in Table 22.

One third of the patients with LHF on admission or during the first day came to hospital within three hours, this is the same as for the whole material. About 40 per cent of the patients with POe were admitted within three hours, a probably significant difference compared with the rest of the material.

Nearly half of the patients with shock came to hospital within three hours, which is significantly higher than for the rest of the material.

Symptoms at onset

Patients with signs of LHF and POe reported, as could be expected, a higher frequency of dyspnoea (Table 22) On the other hand chest pains was experienced less commonly by patients with POe ($p<0.001$) as they were by patients with hypotension or shock ($p<0.001$ and $p<0.01$)

Dyspnoea at onset was also a common symptom in patients with hypotension or shock.

Physical findings

The interrelationships of physical findings discussed here are presented in Table 23

It will be seen that there is a clear relationship between LHF or POe and shock. Thus 22 per cent of patients with POe during the first day also had shock and the corresponding figure for patients with LHF was 13 per cent ($p<0.001$) Fourty three per cent of the patients in shock had signs of LHF and 15 per cent had frank pulmonary oedema ($p<0.001$)

TABLE 22. Delay and symptoms at onset in relation to physical findings on admission and during the first day

		On admission				During first day			
	Total	LHF	POe	Hypo-tension	Shock	LHF	POe	Hypo-tension	Shock
Patients	1827	382	104	91	116	578	146	176	202
Delay (in hours)									
1–3	31%	33%	41%	36%	46%	34%	40%	35%	41%
3–6	18	16	12	17	10	16	13	15	15
6–12	13	11	12	4	6	10	12	11	10
Pain									
no pain	5	7	25	10	10	6	18	9	10
pain>30 min	79	75	47	64	60	78	60	70	66
Dyspnoea	41	67	97	57	54	55	87	48	54

One hospital excluded

TABLE 23. Interrelationship of left heart failure pulmonary oedema hypotension and shock during the first day.

Physical findings	Total	LHF	POe	Hypotension	Shock
			Physical findings		
Patients	1827*	578	146	176	202
LHF	31%			38%	43%
POe	8			6	15
Hypotension	8	10%	11%		
Shock	11	13	22		

One hospital excluded

TABLE 24 Arrhythmias in relation to physical findings during the first day

Arrhythmias during first day	Total	LHF	POe	Hypotension	Shock
		Physical findings during first day			
Patients	1650	339	96	76	104
A V block I	7%	11%	13%	12%	6%
A V block II	5	7	6	10	10
A V block III	6	10	3	11	21
LBBB	10	9	30	19	22
RBBB	5	8	2	11	12
SVT	15	37	51	41	42
SVB	11	12	3	16	16
SVES	24	27	23	21	22
AF	12	19	19	18	16
VEB (>5/min)	22	29	25	30	20
VT	14	20	25	18	27
VF	5	7	12	11	15
Asystole	11	15	17	17	41

Three hospitals excluded.

Arrhythmias during the first day

In Table 24 arrhythmias during the first day are related to physical findings as LHF and POe hypotension and shock during the same time. Three hospitals excluded (see page 22 and 23). This table shows, that these patient-groups as a rule had higher incidence of different arrhythmias compared to the whole material.

Patients with LHF had a significantly higher incidence of SVT AF frequent VEB and VT than for the rest of the material. The same could be observed for patients with POe but these patients also had a high incidence of LBBB the difference being highly significant than for the rest of the material. The incidence of A V conduction defects among these patients did not differ significantly from other patients.

Patients with hypotension and shock also showed a higher incidence of different arrhythmias, especially of A V block III LBBB SVT and VT. They also had a significantly higher incidence of SVB than for the rest of the material, in contrast to patients with LHF and POe

Comments

Many authors have classified patients with MI by the clinical picture (Rosenbaum and Levine 1941 Helander 1949 Willgren 1950, Russek and Zohman 1952, Schnur 1953 b Honey and Truelove 1957 Hamagel et al. 1959 Peel et al. 1962 Robinson et al. 1964 Goble et al. 1966 Killip and Kimball 1967 Lawrie et al. 1967 Stock et al. 1967 McLean et al. 1968, Sloman et al. 1968 Thomas et al. 1968). High incidences of serious arrhythmias have been found in severely ill patient groups (Goble et al. 1966 Killip and Kimball 1967 McMillan et al 1967 Stock et al. 1967 Kimball and Killip 1968, Pentecost and Mayne 1968, Eddy and Mackinnon 1970 Wan et al. 1971) but it seems that VF may occur without signs of major cardiac damage (Julian et al. 1964 Goble et al. 1966 Lawrie et al. 1968 Papp 1969).

Some authors have differentiated primary and secondary arrhythmias (Meltzer and Kitchell 1966, Lawrie et al. 1967 Oliver et al. 1967 Sloman et al. 1968 McDonald et al. 1969). This could not be done with any confidence in the present study but the very high incidence of different arrhythmias support earlier experiences that severely ill patients with signs of LHF POe hypotension or shock are prone to life-threatening arrhythmias.

Maximum SGOT values

No distinct tendencies were found between max. SGOT-values and signs of LHF or POe on admission and during the first day

However patients with signs of shock had a tendency to higher max. SGOT-values (>300 units/ml) than patients without shock (20 against 13 per cent, $p < 0.05$).

The site of infarction has been of particular interest in the evaluation of the prognosis in AMI. This section reports the diagnostic changes from admission to the end of the CCU-stay and relates the final ECG-diagnosis to mortality previous diseases, symptoms at onset, physical findings and arrhythmias during the first day

ECG-diagnosis on admission and during CCU-stay

The site of infarction on admission is compared with the final ECG-diagnosis in Table 25 Three kinds of infarction dominate in this material, namely anterior wall (32 per cent) inferior wall (24 per cent) and inconclusive ECG-changes (24 per cent). Anterior wall infarction was significantly more frequent than inferior wall infarction. The inconclusive ECG-changes include bundle ranch block and ECG-changes not fulfilling the CG-criteria. Other sites of infarction made up about 15 per cent. No sign of infarction, which was found in 10 per cent on admission had decreased to 5 per cent at the end of the CCU stay and "uncertain" infarctions were reduced from 38 to 24 per cent.

Of the patients with anterior MI 72 per cent had ECG-changes on admission which permitted a definite localization on that occasion 6 per cent were diagnosed as no infarction and 21 per cent as uncertain. The corresponding figures for inferior wall infarction were 74 4 and 20 per cent.

On admission, 206 patients had ECG-recordings which were judged as negative. Of these 18 per cent were finally classified as anterior wall infarction, 10 per cent as inferior wall infarction and 17 per cent were diagnosed as uncertain. Among 756 patients with uncertain ECG findings on admission 18 per cent became anterior 13 per cent inferior wall infarction and 59 per cent were still judged as uncertain at the end of the CCU-stay

Mortality

Mortality is related to site of infarction in Table 26. There were only minor differences in the mortality by infarct localization except in the case of patients with lateral infarction whose hospital mortality was significantly lower than the rest of the patients.

Patients with uncertain ECG findings had an increased mortality both during the CCU-stay and during after-care (23 and 13 per cent respectively), the differences compared with the rest of the material being highly significant.

Comments

Many authors have found that anterior wall MI carries a higher mortality than inferior wall infarc

TABLE 25 Diagnostic changes of infarction site from admission to end of CCU-stay

Site of infarction	Total: On admission	Total: Final	Site of infarction according to final ECG-diagnosis: No sign	Ant.	Lat.	Inf.	Ant.–Lat.	Inf.–Lat.	Comb.	Uncertain
Patients	2008	2008	101	648	89	490	79	73	34	482
No sign of infarction	10%	5%	100%	6%	9%	4%	5%	1%	–	7%
Anterior	25	32		72	–	1	20	–	15	–
Lateral	2	4			46	–	3	4	–	–
Inferior	19	24	–		–	74	1	18	17	–
Antero-Lateral	2	4		–	–	–	56	–	–	–
Infero-Lateral	2	4				–	–	48	9	–
Combined	1	2				–	–	–	44	–
Uncertain	38	24		21	45	20	14	23	15	93

TABLE 26 *Mortality in relation to site of infarction.*

Mortality	Total	No sign	Ant.	Lat.	Inf.	Ant.–Lat.	Inf.–Lat.	Comb.	Uncertain
				Site of infarction					
Patients	2008	101	648	89	490	79	73	34	482
CCU	16.1%	18%	13%	9%	15%	19%	8%	18%	23%
After-care	10.5	12	10	5	9	11	4	6	14
Hospital	26.6	30	23	14	24	30	12	24	37

tion (Jacobs 1951 Smith and Denham 1951 Burstein 1953 Seldon 1955 Harnagel et al. 1959 Rosenberg and Malach 1960; Bailey and Beaven 1968; Friedberg et al. 1968, Lassers and Julian 1968 Isomaki et al. 1969 Lemberg et al 1971) Norris et al. (1969 a) differentiated transmural and subendocardial infarction and found that the prognosis for anterior transmural infarction was worse than for inferior transmural but that there was no significant difference between anterior and inferior subendocardial infarctions. Many authors found no significant differences in the prognosis between these two sites of myocardial infarction (Master et al. 1939 Rosenbaum and Levine 1941 Woods and Barnes 1942; Mintz and Katz 1947 Billings et al. 1949 Doscher and Poindexter 1950 Willgren 1950; Lindén 1952, Beard et al. 1960 Imperial et al. 1960) and others report a higher mortality for inferior infarctions. (Nylin and Ejrup 1943· George and Greenwood 1967)

A better short term prognosis has been reported for patients with lateral infarction (Katz et al 1949 Binder and Sbertoli 1955) an this was the case in the present study Patients with infarction extending over more than on surface of the heart had been found to have an increased mortality (Mintz and Katz 1947 Seldon 1955 Harnagel et al. 1959· Stock 1967) but this was not so in the present study

High mortality has been reported for patients with ECG-changes classified as obscure or indefinite (Rosenbaum and Levine 1941 Woods and Barnes 1942, Selzer 1948, Doscher and Poindexter 1950; Binder and Sbertoli 1955 Isomaki et al. 1969· Norris et al. 1969 a). The present findings are in line with this but it should be added that the high mortality in these patient groups probably has to do with factors that make the ECG assessment uncertain, e.g. bundle branch block, tachycardia, earlier infarction, left heart failure shock etc.

Previous diseases

Patients with uncertain ECG findings had a higher incidence of previous diseases such as chronic angina pectoris, earlier myocardial infarctions, left heart failure and diabetes (Table 27). The differences from the rest of the material are highly significant. Patients with no ECG signs of MI showed the same trend as patients with inconclusive ECG-changes.

Patients with anterior wall infarction had a lower incidence of chronic angina pectoris, earlier myocardial infarction and congestive heart failure. The differences from the rest of the material are highly significant. The same trend was found for patients with inferior wall infarction.

Symptoms at onset

Symptoms at onset in relation to site of infarction are shown in Table 28.

In the case of chest pain, there were small differences between infarction sites However only 59 per cent of the patients with uncertain ECG-recordings mentioned chest pains for more than 30 minutes, which is significantly less ($p<0.001$) than in the rest of the material.

About 40 per cent of the patients with a positive ECG-diagnosis mentioned dyspnoea at the onset of symptoms, as against 52 per cent of patients with uncertain ECG-recordings. The difference is highly significant.

A history of dizziness and/or fainting and/or syncope was reported in about 18 per cent of the patients, with no significant differences between

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Comments

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TABLE 25 Diagnostic changes of infarction site from admission to end of CCU-stay

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Patient	2008	2008	101	648	89	490	79	73	34	482
No sign of infarction	10%	5%	100%	6%	9%	4%	5%	1%	–	7%
Anterior	25	32		72		1	20	–	15	–
Lateral	2	4		–	46	–	3	4	–	–
Inferior	19	24		–		74	1	18	17	–
Antero-Lateral	2	4	–				56	–	–	–
Infero-Lateral	2	4	–			–		48	9	–
Combined	1	2				–	–	–	44	–
Uncertain	38	24		21	45	20	14	23	15	93

as nausea and vomiting were equally common in anterior and inferior myocardial infarctions and the findings accordingly do not support the theory that these symptoms are an effect of a local process in the inferior wall of the myocardium.

Physical findings

Physical findings during the first day are related to the site of infarction in Table 29

Anterior wall infarction carried a significantly higher incidence of LHF than inferior wall infarction, whereas the latter had a significantly higher incidence of hypotension ($p<0.001$).

Patients with uncertain ECG findings had higher incidence of LHF POe hypotension and shock than the rest of the material and these differences are highly significant.

Arrhythmias during the first day

The incidence of arrhythmias during the first day is related to different sites of infarction in Table 30 Patients with anterior wall infarction had higher incidences of LBBB SVT and frequent VEB than those with inferior wall infarcts. The latter on the other hand had higher incidences of A V blocks of all degrees and SVB

High incidences of different arrhythmias, especially LBBB SVT and atrial fibrillation, were found, as might be expected, in patients with inconclusive ECG-diagnosis.

TABLE 29 Physical findings during the first day in relation to site of infarction according to ECG

Physical findings	Site of infarction								
	Total	No sign	Ant.	Lat.	Inf.	Ant. Lat.	Inf.–Lat.	Comb	Uncertain
Patients	2008	101	648	89	490	79	73	34	482
Left heart failure	28%	26%	29%	28%	20%	38%	19%	32%	36%
Pulmonary oedema	5	9	2	3	2	3	1	–	11
Hypotension	7	6	4	3	9	6	14	9	10
Shock	8	9	6	5	7	9	4	9	12

TABLE 30 Arrhythmias during the first day in relation to site f infarction

Arrhythmias	Site of infarction								
	Total	N sign	Ant.	Lat.	Inf	Ant. Lat.	Inf.–Lat.	Comb.	Uncertain
Patients	1831	93	595	75	441	73	73	32	439
A–V block I	6%	2%	4%	1%	8%	4%	11%	9%	8%
A V block II	4	2	2	3	8	4	12	12	3
A V block III	5	2	3	–	8	3	10	19	5
LBBB	9	14	6	4	3	7	–	–	21
RBBB	5	1	5	4	3	1	6	3	7
SVT	21	18	21	16	15	26	19	25	29
SVB	9	5	6	5	15	15	22	19	6
SVES	23	19	22	23	22	16	21	9	29
AF	11	17	8	7	9	11	4	6	18
NR	7	7	6	5	8	10	8	–	8
VES (>5/min)	16	15	17	12	12	16	15	9	20
VT	13	11	10	7	10	21	15	19	19
VF	7	3	6	9	7	6	10	6	9
Asystole	10	12	10	7	10	8	8	12	13

Two hospitals excluded, (See page 23)

The continuous monitoring of patients with acute myocardial infarction has increased the knowledge of arrhythmias and their prognostic importance during the early stage of this disease. In the present study different arrhythmias have been analysed in relation to most of the available data. This section deals with the relation to mortality previous diseases, delay symptoms at onset, physical findings and SGOT-maximum. The interrelationship of different arrhythmias is also analysed.

Incidence

The incidences of different arrhythmias on admission and during the first day are shown in Tables 8 and 9 respectively. The short ECG-registration on admission agreed surprisingly well with the findings during the first day at least for LBBB (8 and r cent), RBBB (4 and 5 per cent) and AF (9 12 per cent). As one would expect higher idences were registered during the first day for rrhythmias as a sign of electrical instability i.e. SVEB (6 and 24 per cent), frequent VEB (6 and 21 per cent) and VT (2 and 13 per cent).

Comments

The incidences of most common arrhythmias in the present study are well in line with many other reports (Julian et al. 1964, Meltzer and Kitchell 1966, Killip and Kimball 1967, Lawrie et al. 1967, Lown et al. 1967 a, Morsey 1967, Stock et al., 1967, Marshall et al. 1968, Mogensen 1970).

Even so, the present figures must be regarded as rather approximate and be interpreted very cautiously. Many factors are involved in determinations of these incidences. The equipment was by no means the same at the various hospitals; for instance two hospitals had to be excluded when calculating incidence figures of arrhythmias during the first day in CCU because they lacked the facilities for continuous monitoring of every patient throughout the CCU stay (Tables 31–36).

The observer variation in a multicenter study like this must vary among other things with the training and skill of the CCU staff.

In general it was not possible to use more advanced methods for differentiating between arrhythmias. The difficulty of differentiating VEB from SVEB with aberrant conduction is well-known and with the present criteria for premature beats (page 11) many SVEB with aberration were probably classified as VEB.

The frequencies for different arrhythmias given here are no doubt on the low side especially for transient arrhythmias such as SVEB, VEB and short episodes of VT. In an evaluation of continuous ECG recordings in a coronary care unit, Mogensen (1970) reported that VT had been detected at the time in approximately 50 per cent of those patients for whom the retrospective analysis revealed one or more episodes of VT. In their evaluation of a new antiarrhythmic drug Rydén and co-workers (1974) came to a similar conclusion concerning the accuracy of nurse-based arrhythmia detection in a CCU.

Mortality

The mortality in relation to arrhythmias on admission and during the first day is shown in Tables 31 and 32 respectively.

Patients with A V block and bundle branch block had a very high mortality during both the CCU-stay and the total hospital period.

Compared to the rest of the patients, the differences are highly significant. A high hospital mortality (40 per cent) was also found for patients with arrhythmias indicating LHF i.e. SVT and AF ($p<0.001$). The patients with ventricular arrhythmias (frequent VEB and VT) also had a high mortality (32 and 42 per cent).

The only arrhythmias analysed here that were not associated with an increased mortality were SVB and SVEB. However 129 patients developed SVB during the first day and these patients had a significantly higher mortality during the CCU-stay.

Comments

It has been well-known for many years that patients with arrhythmias have a poorer prognosis

TABLE 31. Mortality in relation to arrhythmias on admission

Mortality	Total	Arrhythmias on admission: A–V I	A–V II	A–V III	LBBB	RBBB	SVT	SVB	SVEB	AF	VEB	VT
Patients	1831	48	23	40	142	67	208	61	103	155	116	37
CCU	15.8%	21%	26%	30%	32%	31%	26%	13%	18%	23%	26%	30%
After-care	10.6	14	2	8	16	12	20	3	8	16	16	13
Hospital	26.4	35	48	38	48	43	46	16	26	39	42	43

Two hospitals excluded

TABLE 32. Mortality in relation to arrhythmias during the first day

Mortality	Total	Arrhythmias during the first day: A–V block I	A–V block II	A–V block III	LBBB	RBBB	SVT	SVB	SVEB	AF	VEB	VT	VF
Patients	1831	121	84	102	171	93	435	190	447	225	375	246	88
CCU	15.8%	29%	37%	46%	32%	31%	24%	22%	16%	22%	22%	30%	67%
After-care	10.6	14	12	8	16	13	16	7	9	18	10	12	9
Hospital	26.4	43	49	54	48	44	40	29	25	40	32	42	76

Two hospitals excluded

than those without (Master et al. 1937 Rosenbaum and Levine 1941 Binder and Sbertoli 1955 Honey and Truelove 1957 Johnson and Minor 1958, Beard et al. 1960, Hurwitz and Eliot 1964 Bailey and Beaven 1968) but it is still very difficult to evaluate the prognostic significance of a particular arrhythmia because the occurrence of some arrhythmias is mostly associated with the clinical state, especially severe heart failure hypotension and shock (Goble et al. 1966 Meltzer and Kitchell 1966 Killip and Kimball 1967 Weil and Shubin 1968, Lown et al. 1969). The abnormal rhythm may not always be the cause of death. Consequently the prognostic importance of different arrhythmias should always be evaluated in relation to other clinical findings (Paulk and Hurst 1966, Fluck et al. 1967 Stock et al. 1967).

The mortality figures given in the present study make no allowance for whether the arrhythmia was primary or secondary or whether it was the real cause of death.

The high mortality among patients with atrioventricular conduction defects is in keeping with many studies and of about the same largeness as the present one (Paulk and Hurst 1966 Day 1968 Friedberg et al. 1968 Brown et al. 1969 Bloomfield et al. 1970 Eddy and Mackinnon 1970 Mogensen 1970 Sloman et al 1971). The grave prognosis with complete heart block at anterior infarction has been pointed out in particular (Friedberg et al 1968 Norris 1969 c Lemberg et al. 1971 Watson and Goldberg 1971).

The same high mortality for bundle branch block as in the present study is likewise reported in the literature (Master et al 1937 Imperial et al 1960 Hipp et al 1961 Julian et al 1964 Bauer et al 1965 Day 1968 Hunt and Sloman 1969 Godman et al 1970 Mogensen 1970 Norris and Croxson 1970).

The study shows no significant difference in mortality between LBBB and RBBB this is in accordance with certain reports (Hunt and Sloman 1969 Godman et al 1970) but others indicate the mortality for RBBB is somewhat higher than for LBBB (Mogensen 1970 Norris and Croxson 1970). Some authors also report that patients with bundle branch block were older and had a higher incidence of pre-existing heart diseases than other

patients (Hipp et al. 1961 Bauer et al. 1965) which is well in line with the present findings.

The high mortality for patients with SVT and AF is probably a sign of LHF which could help to explain the high mortality figures for these arrhythmias. Many other studies have shown an increased mortality for patients with SVT (Binder and Sbertoli 1955 Scherf 1958 Hurwitz and Eliot 1964 Jewitt et al. 1967 Kimball and Killip 1968 Bloomfield et al. 1970 Mogensen 1970) and for these with AF (Billings et al. 1949 Jewitt et al. 1967 Bailey and Beaven 1968 Klass and Haywood 1970 Mogensen 1970 Helmers et al. 1973). Askey and Neurath (1945) and Binder and Sbertoli (1955) particularly pointed out the poorer prognosis for persistent AF

The importance of SVB is a controversial subject. Some authors have not found SVB to be associated with any increased mortality (Scherf 1958 Imperial et al. 1960 Hurwitz and Eliot 1964 Julian et al. 1964 Fluck et al 1967 Lawrie et al 1967 Lown et al 1967 a) others report a poorer prognosis in patients with AMI and SVB ...ings et al. 1949 Binder and Sbertoli 1955 ... et al. 1963 Lemberg et al. 1971) Adgey et al. (1968) found a high incidence of SVB when the patients were seen early (within 4 hours with mobile CCU) after the onset of AMI, especially in association with posterior infarction. SVB could be an important precursor of VF and an important factor in the high early mortality from AMI The low mortality for SVB in some materials might be explained by longer delay time. The present findings of a higher mortality for patients who developed SVB during their CCU stay but not for patients with SVB as a whole partly supported these opinions.

Ventricular arrhythmias both as VEB (frequent, multifocal, paired and R on T type) and especially VT are associated with increased mortality in most studies (Binder and Sbertoli 1955 Hurwitz and Eliot 1964 Julian et al. 1964 Cohn et al. 1966 Dalle et al. 1967 Lawrie et al. 1967 Stock et al. 1967 Raftery et al. 1969 Mogensen 1970 Sloman et al. 1971) findings which are confirmed in the present study

Previous diseases

The relationships between previous diseases and arrhythmias during the first day are shown in Table 33

Patients with *A V block I* had higher frequencies of chronic AP and a positive history of CHF than the rest of the material. The difference is probably significant for AP and highly significant for CHF

Only 14 per cent of the patients with *A V block III* during the first day had had one MI before and

TABLE 33 Previous diseases in relation to arrhythmias during the first day

Previous diseases	Total	A V block I	A V block II	A V block III	LBBB	RBBB	SVT	SVB	SVEB	AF	VEB	VT	VF
Patients	1831	121	84	103	171	93	435	190	447	225	375	246	88
Angina pectoris													
None	35%	31%	42%	47%	29%	39%	33%	45%	40%	35%	38%	39%	33%
<1 month	22	22	19	15	16	27	21	19	19	19	17	16	11
1 6 months	9	7	1	9	8	6	6	7	9	8	10	7	8
>6 months	[illegible]	36	33	26	40	20	34	24	28	33	31	34	38
Prev myocard inf													
None	64	64	61	75	52	70	59	69	64	68	67	63	47
One	26	26	25	14	30	20	28	31	25	20	22	23	35
Two or more	8	8	10	8	15	8	10	6	9	9	10	11	10
CHF	24	47	29	26	53	27	29	20	29	52	33	34	24
Hypertension	25	24	25	20	29	19	31	24	28	28	30	24	19
Diabetes	12	17	13	16	19	8	14	5	10	18	13	10	9

Two hospitals excluded

this is significantly less than in the rest of the material.

A large proportion of the patients with *LBBB* had chronic AP (40 per cent) two or more infarctions earlier (15 per cent) previous CHF (53 per cent) and diabetes (19 per cent) the differences are highly significant compared with patients without these ECG changes.

Patients with *SVT* had significantly higher incidences of chronic AP ($p<0.001$), CHF ($p<0.01$) and hypertension ($p<0.001$).

Patients with *SVB* had a low frequency of diabetes ($p<0.01$). The large group of patients with *SVEB* had a high frequency of CHF (29 per cent) compared with the rest of the patients ($p<0.01$).

The incidences of CHF and diabetes were higher among patients with *AF* the differences being highly significant compared with patients without this arrhythmia.

Patients with frequent *VEB* had a higher incidence of CHF and this difference is highly significant.

Significantly higher frequencies of chronic AP ($p<0.01$) and CHF ($p<0.001$) were noted in patients with *VT* during the first day

There were 88 patients who developed *VF* during the first day and the frequency of chronic AP and one infarction earlier was significantly higher among them then in the rest of the material.

Delay

Delay is related to arrhythmias during the first day in Table 34 About 45 per cent of the patients with A V block III *SVB* frequent *VEB* and *VT* during the first day arrived at hospital within three hours after the onset of symptoms. This is significantly higher ($p< 0.001$) than the rate for the rest of the material. Otherwise the length of the delay did not differ significantly between patients with different kinds of arrhythmias.

Symptoms at onset

Symptoms at onset in relation to arrhythmias are shown in Table 35 A high frequency of dyspnoea (61 per cent) was noted in patients with *LBBB* ($p<0.001$), as it was in patients with *RBBB* though this is only probably significant. Patients with *SVT* also had a significantly higher frequency of dyspnoea (55 per cent) compared with the rest of the material ($p<0.001$), as did patients with *AF*

Physical findings

Physical findings during the first day are related to arrhythmias during that time in Table 36. Three hospitals excluded (see page 22 and 23).

High frequencies of LHF were observed in all the arrhythmia groups listed in the table Only for *SVB* there was no significant difference compared with the rest of the material. The patients with *LBBB* and *SVT* also had high incidences of POe.

In general the same trends were found for the different arrhythmias in relation to hypotension and shock. It is of interest that the 190 patients with *SVB* had significantly higher frequencies for hypotension and shock (16 and 15 per cent) but not for LHF and POe, than for the rest of the material.

TABLE 34 Delay in relation to arrhythmias during the first day

Delay time in hours	Total	A–V block I	A–V block II	A–V block III	LBBB	RBBB	SVT	SVB	SVEB	AF	VEB	VT	VF
Patients	1831	121	84	102	171	93	435	190	447	225	375	246	88
1 3 hours	31%	31%	33%	43%	33%	27%	33%	46%	33%	30%	44%	47%	38%
4–6 hours	17	25	21	14	15	20	16	16	19	20	19	16	19
6–12 hours	13	12	8	10	15	12	14	14	12	8	10	9	6

Arrhythmias during the first day (column group heading spanning A–V block through VF)

Two hospitals excluded

TABLE 35 Symptoms at onset in relation to arrhythmias during the first day

Symptoms at onset	Total	Arrhythmias during the first day: A–V block I	II	III	LBBB	RBBB	SVT	SVB	SVEB	AF	VEB	VT	VF
Patients	1831	121	84	102	171	93	435	190	447	225	375	246	88
No pain	5%	7%	7%	12%	9%	4%	6%	4%	6%	10%	8%	7%	7%
Pain <30 min	17	24	22	16	24	22	18	18	18	19	17	22	20
Pain >30 min	68	58	58	62	56	60	65	70	68	59	68	63	56
Dyspnoea	42	52	45	44	61	53	55	38	46	56	43	44	48

Two hospitals excluded

TABLE 36 Physical findings in relation to arrhythmias during the first day

Physical findings	Total	Arrhythmias during the first day: A–V block I	II	III	LBBB	RBBB	SVT	SVB	SVEB	AF	VEB	VT	VF
Patients	1650	120	76	95	168	87	416	187	397	205	369	233	77
LHF	31%	48%	50%	43%	49%	41%	47%	32%	37%	48%	41%	50%	51%
Pulm. oedema	5	8	4	4	17	5	13	5	6	8	7	11	16
Hypotension	8	15	21	18	14	9	12	17	8	11	13	15	7
Shock	9	13	17	26	21	16	15	15	9	11	13	22	43

hree hospitals excluded

Maximum SGOT

No correlation was found between the different arrhythmia groups and maximum SGOT values

Interrelationship of arrhythmias

The interrelationship of different arrhythmias is shown in Table 37 It will be seen that patients with one form of arrhythmia during the first day in CCU also had *many other kinds of arrhythmias* during this time Only the most striking findings will be pointed out here

Patients with A V block I had higher incidences of other atrioventricular conduction defects, with highly significant differences compared to the rest of the material The incidence of LBBB (23 per cent) was also significantly higher ($p<0.001$) but not that of RBBB These patients also had a tendency to develop ventricular dysrhythmias as frequent VEB (36 per cent), this difference was highly significant.

Of the patients with A V block II 43 per cent showed periods of A V block III during the first day Patients with A V block II also had high incidences of SVT (38 per cent) and SVB (26 per cent) ($p<0.001$ and $p<0.01$ respectively). Furthermore *these patients showed significantly higher* incidences of VT (32 per cent) and VF (16 per cent) during the first day ($p<0.001$)

Patients with complete heart block had high incidences of SVB, VT and VF (25 28 and 15 per *cent). The same relationship was found with* LBBB and RBBB.

Patients with LBBB had significantly higher incidences of SVT and AF 44 and 25 per cent respectively ($p<0.001$). Even ventricular dysrhythmias were more common among these patients. Besides the above-mentioned relation to A V block III, patients with RBBB had significantly increased incidences of SVT and SVB (37 and 18 per cent) compared with other patients.

Patients with SVB had significantly higher incidences of A V blocks II and III VEB NR and VT ($p<0.001$).

TABLE 37 Interrelationship of arrhythmias during the first day

Arrhythmias during the first day	Total	A–V block I	II	III	LBBB	RBBB	SVT	SVB	SVEB	AF	VEB	VT	VF
Patients	1831	121	84	102	171	93	435	190	447	225	375	246	88
A–V I	7%		46%	25%	16%	10%	10%	10%	8%	7%	12%	10%	14%
A–V II	5	32%		35	7	5	7	12	6	4	6	11	15
A V III	6	21	43		9	12	6	13	4	7	9	12	17
LBBB	9	23	14	14		7	18	10	11	19	14	19	18
RBBB	5	7	6	11	4		8	9	6	4	7	6	7
SVT	24	35	38	28	44	37		27	29	32	32	39	41
SVB	10	16	26	25	11	18	12		13	8	16	20	14
SVEB	24	30	31	18	29	27	30	31		26	34	34	21
AF	12	12	11	16	25	10	16	10	13		17	18	18
VEB <5 per min	37	35	40	27	38	31	36	38	47	43		44	34
VEB >5 per min	21	36	29	31	30	28	27	31	29	28		45	34
VT	13	20	32	28	27	15	22	25	19	19	29		55
VF	5	10	16	15	9	7	8	6	4	7	8	20	
Asystole	10	20	26	34	19	19	14	20	10	12	16	24	67

Two hospitals excluded

Patients with AF had, in addition to the relation to LBBB, high incidences of SVT frequent VEB and VT (32, 28 and 19 per cent)

Patients with frequent VEB or VT had a very high incidence of almost all the forms of arrhythmias listed in Table 37 only for A V block II and RBBB there was no significant increase.

The development of special apparatus and techniques for the treatment and care of patients with acute myocardial infarction has lead to the establishment of coronary care units (CCU) Evaluative studies of such units started to appear around the mid-Sixties (Brown et al. 1963 Julian et al. 1964 Day 1965). Favourable results were obtained chiefly with the active treatment of certain arrhythmias, perhaps mainly through the early institution of prophylactic therapy against ventricular ectopic beats with a view to preventing the development of more serious arrhythmias, pri marily ventricular tachycardia and ventricular fibrillation (Lown et al. 1967 a, Killip and Kimball 1968).

Experience of CCUs at hospitals in Sweden was not very extensive during the latter part of the Sixties. Setting up such units involves heavy investment in technical equipment and staff. In 1968 the Swedish Society of Cardiology therefore tiated a multi-center study of patients admitted CCUs, the aim being to appraise the value organization and design of such units at an early stage and to obtain as detailed a picture as possible of patients with AMI.

One advantage of co-ordinating the care and treatment of a uniform group of patients at several hospitals was that within a relatively short time would be able to collect a sufficiently large number of cases to permit a breakdown into sub-groups for closer assessments and prognostic evaluation.

The study was prospective and the data were collected in a uniform manner The amount of information that was assembled during the patients stay at the CCUs was very large

So many hospitals participated that the study covered a relatively large proportion of infarct patients admitted to hospital in Sweden and it can possibly be regarded as representative of the entire country during the year in question.

A study of this type presents considerable difficulties. Assessments are complicated by diffe rences in the populations of the various hospital areas, varying distances to the hospitals, previous policies for the care of persons with acute chest pains and differences in the age of patients from different hospitals. Although an attempt was made from the start to apply uniform regulations for the admission of patients to CCUs, local conditions often intervened. Some hospitals accordingly applied an upper age limit but even so, a number of "older" patients were admitted to their CCU mostly on the indication of critical arrhythmia. The difficulty of applying uniform regulations for admission to the various hospitals should also be mentioned. Although responsibility for admitting and treating patients at the CCU of each hospital rested with a small group of physicians, the duty system meant that many other physicians took over this responsibility at times, particularly at night.

Another difficulty concerns the uniform application of definitions in the assessment and classification of clinical states. Part of the problem lies in the amount of time and interest which different physicians were able to devote to the study By way of illustration it can be mentioned that left heart failure was reported for as many as 66 per cent of the patients from hospital but for only about 20 per cent of those from several other hospitals.

The skill and training of nurses and other staff no doubt played a considerable part, too. Training was however uniform in that it was based on a single manual (Lundman et al. 1968 a) but there was no doubt a relatively wide variation in the observer variation. There were also considerable differences in equipment and facilities for monitoring which would likewise influence the results, chiefly perhaps the diagnosis of arrhythmias. This was why two hospitals had to be excluded from the section on arrhythmias.

Many factors thus contribute to the highly heterogeneous nature of the material collected in the present study and one must therefore be rather cautious when assessing the results. Bu factors have been found to have a beari short term prognosis for patients with A

By collecting information about

computerized records it is possible to process a vast quantity of facts. It used to be argued that many problems would be solved once information about the patients had been collected and processed but more recent assessments are less optimistic in this respect (Baird and Garfunkel 1965 Barnett 1968, Thompson et al. 1971). The general documentation of data with a view to some future, possibly scientific usefulness is meaningless — "The documentation of thousands and thousands of items without having a scientific question in mind and without knowing for what purpose the data is collected will lead to nothing. This is documentation of nonsense" (Wagner 1970)

Notwithstanding the very heterogeneous nature of the material, the difficulties in assessing certain variables (chiefly the clinical findings and the diagnosis of arrhythmias) and the low validity in certain respect (chiefly due to large differences in observer variation) it was nevertheless considered that a more extensive processing of the data might be of value. The material could provide a basis for a prognostic assessment and permit the evaluation of earlier prognostic indices. The results can also be used as reference material and for further follow-up studies.

The major influence of age on mortality is clearly indicated by the present results. Mortality was relatively low and fairly constant up to the age of 55–60 years and rose considerably more steeply after 60–65 years. But age also proved to be important in several other respects, a relationship being found with such variables as earlier diseases, physical findings and certain forms of arrhythmia, chiefly LBBB and AF

The composition of infarction materials by age should therefore be indicated clearly and age must also be taken into account when evaluating other variables, particularly in terms of prognosis. Age is likewise an important factor in the assessment of individual cases, partly because elderly persons are often affected by disorders in other vital organ systems, so that acute infarction may be a manifestation of some other complaint.

The variables for which no definite correlation with age was found were chiefly delay site of infarction and most forms of arrhythmia.

The study suggests that young patients come to hospital more quickly than older ones. Similar results have been reported by for instance Hacket and Cassem (1969) and Moss et al. (1969).

There is possibly a tendency for inferior infarction to be somewhat more common among the younger groups of patients but no definite conclusions can be drawn about this. It is quite clear that the proportion of uncertain and indefinite ECG recordings increased with age partly because the older groups had an increased incidence of other ECG changes, chiefly LBBB and AF

The occurrence of A V block I also appears to increase with age though this may be an effect of medication, mainly with digitalis, for earlier or incipient signs of left ventricular failure. In other words, the increased incidence of A V block I among the older groups in this study may not be correlated directly with age

The variations in the interval between onset of symptoms and admission to hospital seem to have had relatively little influence on the mortality in this material.

There is possibly a tendency for patients in a major urban area to come to hospital with less delay than those in other parts of the country Some part is no doubt played by geographical conditions, particularly the large differences in the distance to hospital and to some extent perhaps the differences in ambulance services and access to these, but in any event the differences were not large.

The validity of the information about the interval between onset of symptoms and admission to hospital is for various reasons too low to permit any definite conclusions about the influence of the delay on mortality or about comparisons between age groups and between geographical areas.

Patients with certain earlier diseases had a considerably higher mortality than those without a history of these disorders. This applied in particular to patients with earlier symptoms of chronic angina pectoris, earlier cardiac insufficiency and patients with diabetes mellitus. But as it is chiefly older individuals who are afflicted with these diseases, it is very difficult to evaluate the prognostic importance of these diseases by themselves.

Patients with a history of chronic disease also displayed a higher incidence of other chronic disorders than the rest of the material. In particular patients with CHF presented high incidences

of angina pectoris and previous infarction.

Patients with a failing pump action had a high incidence of life threatening arrhythmias, chiefly A V block III, LBBB SVT and VT It was not possible to determine the extent to which these arrhythmias had contributed to the impairment of pump action but in many cases they were secondary to this. There was no possibly of distinguish ing primary arrhythmias from secondary in these patient categories.

Mortality did not differ significantly between patients with an anterior as opposed to an infe rior site of infarction. Patients with ECG findings that were indeterminate had a considerably higher mortality than the rest of the material no doubt as a consequence of several concurrent factors as higher age previous infarction and bundle branch block. The mortality was also somewhat increased in the group with negative ECG i.e. no signs of acute infarction. Many of those ECG recordings were probably likewise difficult to assess and were classified as negative with respect to acute infarc tion. These and the indeterminate group may be similar in several respect.

Most forms of arrhythmia were associated with a considerably increased mortality Only SVB and SVEB differ in this respect. The highest mortality rates were found for patients with such arrhythmias as A-V block II and III, LBBB and VF

As several forms of arrhythmia often occurred together it is difficult to evaluate the prognosis for each form separately

SUMMARY

The aim of the present multicenter study was to investigate the early stage of acute myocardial infarction (AMI).

The material was collected from twelve Swedish hospitals. It comprised 2 008 patients, with verified diagnosis of AMI being treated in the respective CCUs during 1969

Uniform rules were applied to the selection of patients, criteria for admission and diagnosis, definitions of physical findings, principles of treatment and registration of data. The hospitals had agreed to the same data form and this made transformation to punched cards possible and also centralized calculation on a computer

Of the 2 008 patients, 1 447 were men (72.1 per cent) and 561 women (27 9 per cent) with a male/female quotient of 2.6 The mean age for the whole material was 65 5 years, for men 63 8 and for women 69 8 years respectively

The mortality during the CCU-stay was 16.1 per cent and the hospital mortality 26.6 per cent. The mortality was relatively low and constant, about 10 per cent, up to about 50–55 years; after the age of 60–65 years it rose steeply The mortality during the CCU-stay was 14.3 per cent for men and 20.7 per cent for women, and hospital mortality for men was 23 8 and for women 34 0 per cent. The difference between men and women was significant for hospital mortality but there were no significant differences between sexes when age was taken into account.

Chronic angina pectoris and congestive heart failure were more common along with age There was also a similar age correlation for hypertension and diabetes, but this trend was less obvious.

The interval between onset of symptoms and admission to hospital (delay time) was shorter in younger patients. Patients in major urban areas seemed to be admitted more rapidly than those living in county regions but the data cannot support firm conclusions.

Chest pain was the most dominate symptom at onset, especially in younger patients, while dyspnoea was more common in the elderly

Findings such as disturbed consciousness, signs of left heart failure and frank pulmonary oedema, were more common along with age. The incidence of shock tended to increase with age at least among patients over 70 while no age correlation was found for hypotension.

A positive age correlation was noted for left bundle branch block and atrial fibrillation but not for ventricular arrhythmias.

A significantly higher mortality was observed among patients with chronic angina pectoris, positive history of congestive heart failure and diabetes. Patients with a short history of angina pectoris (less than six months) those with one previous infarction or with hypertension did not show significantly increased mortality

Delay time was less than three hours for 30 per cent of the patients, less than six hours for 47 per cent and less than twelve hours for 60 per cent. Delay time was not associated to increased mortality in this patient series.

High mortality rates were noted for patients with signs of left heart failure and frank pulmonary oedema, 40 and 50 per cent respectively and for those in shock, 80 per cent. These physical findings occurred more often in patients with a previous history of ischaemic heart disease. The incidence of arrhythmias was also higher among these patients.

There was no significant difference in mortality between anterior and inferior myocardial infarction, However the mortality was higher in patients where the site of the infarction could not be assessed due to bundle branch block or previous infarction.

The majority of registered arrhythmias was associated with a high mortality Patients with atrioventricular conduction disturbances, bundle branch block supraventricular tachycardia, ventricular ectopic beat and ventricular tachycardia had a mortality of about 40–45 per cnet. Those with supraventricular bradycardia and supraventricular ectopic beat did not show increased mortality (16 and 26 per cent, respectively).

Arrhythmias were highly intercorrelated especially in atrioventricular conduction disturbances.

Of the 84 patients with A-V block II, 25 per cent had A V block I and 35 per cent A V block III. The incidence of supraventricular tachycardia was likewise high in patients with atrioventricular conduction defects as well as for those with bundle branch block. Patients with A V block II and III and with left bundle branch block also had high incidences of ventricular tachycardia. A high incidence of frequent ventricular ectopic beat, more than five per minute was found in patients with ventricular tachycardia.

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PART II

The short-term prognosis

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CONTENTS

ABBREVIATIONS

AF	Atrial fibrillation
AID	Automatic interaction detector
AMI	Acute myocardial infarction
A-V block I	Atrioventricular block I
A-V block II	Atrioventricular block II
A-V block III	Atrioventricular block III or complete heart block
CCU	Coronary care unit
CHD	Coronary heart disease
CHF	Congestive heart failure
DBP max	Maximum diastolic blood pressure
ECG	Electrocardiogram
HR max	Maximum heart rate
IHD	Ischaemic heart disease
LBBB	Left bundle branch block
LHF	Left heart failure
RBBB	Right bundle branch block
RR max	Maximum respiratory rate
SBP min	Minimum systolic blood pressure
SGOT max	Maximum serum glutamate oxaloacetate transaminase
SGPT max	Maximum serum glutamate purvate transaminase
SR	Sedimentation rate
SVB	Supraventricular bradycardia
SVEB	Supraventricular ectopic beats
SVT	Supraventricular tachycardia
VEB freq	Ventricular ectopic beats, frequence (beats per min.)
VF	Ventricular fibrillation
VT	Ventricular tachycardia
WBC	White blood corpuscles

Attempts to predict the short term prognosis of acute myocardial infarction (AMI) are of great importance from the clinical as well as the economic point of view. For clinical purpose a classification in prognostic groups – to create more homogeneous groups of patients – might facilitate the evaluation of results achieved with different principles of treatment.

The management of a coronary care unit (CCU) is expensive. A crucial question is whether a patient can be discharged to a more simple form of treatment or whether he/she belongs to a high-risk group and ought to remain in the unit. An effective prognostic classification might yield more information for the planning and distribution of resources.

Many authors have tried to classify AMI patients on the basis of physical observations (Conner and Holt 1930, Master et al. 1937, Rosenbaum and Levine 1941, Mintz and Katz, 1947, Billings et al. 1949, Helander 1949, Biorck 1957, Honey and Truelove 1957, Harnagel et al. 1959, Beard et al. 1960, Wahlberg 1963, et al. 1964, Lawrie et al. 1968, Sloman et al. 1968). Others have constructed point scales with a view to obtain a more objective prediction of high-risk groups (Peel et al. 1962). Computer techniques and modern methods have been used to develop more advanced approaches (Hughes et al. 1963, Lembich 1965, Norris et al. 1969). After the evolution of CCUs, several authors have constructed systems for evaluating the short term prognosis for patients with AMI treated in such units (Antonini et al. 1970, Bullock et al. 1970, McHugh et Swan 1971, Chapman and Grey 1974, Helmers 1974).

In 1969 the Swedish Society of Cardiology initiated a multi-center study from twelve Swedish hospitals of 2 008 AMI patients who had been admitted to a CCU. The aim of the study was:

1. To obtain as detailed a picture as possible of the early stage of AMI.
2. To evaluate the short term prognosis of AMI.
3. If possible to appraise the organization and design of such units.

In Part I the patient series has been described in detail with respect to age, sex, previous diseases, delay time, physical findings, site of infarction, arrhythmias. The present work concerns the attempts to evaluate the short-term prognosis of these 2 008 patients. This involved the use of statistical methods for determining factors which have an important influence on the short term prognosis of AMI. The study is presented as follows:

Chapter one gives the survival rates for different age groups together with a comparison between survivors and deceased for each variable registered.

Chapter two is devoted to a prognostic estimation, using the automatic interaction detector (AID) analysis.

Chapter three presents corresponding prognostic evaluations using linear regression analysis, multiple logistic analysis and isotonic regression analysis.

Patient series

The cases were collected from twelve Swedish hospitals and the series comprises 2 008 patients, who were treated in the respective CCUs during 1969 with the diagnosis of acute myocardial infarction. Uniform rules were applied to the selection of patients, criteria for admission and diagnosis, definition of physical findings, principles of treatment and registration of data. The hospitals used the same data record, thereby permitting a transferral to punched cards and centralized calculation on a computer.

The CCU mortality in this series was 16.1 per cent and the total hospital mortality 26.6 per cent (males 21 per cent, females 34 per cent).

Chapter I

COMPARISON BETWEEN SURVIVORS AND DECEASED

This chapter is chiefly concerned with a comparison between survivors and deceased in order to identify any significant differences between them with respect to single variables, independent of the influence of other contributory factors. A group comparison is also made between patients who died during the CCU-stay and those who died during the after-care. The risk of dying during the stay in hospitals is studied, too.

Statistical methods

The Chi-square test was used for testing the significance of differences between relative numbers for the total hospital stay. The significance levels considered are expressed in the text as significant ($p<0.01$) and highly significant ($p<0.001$).

To describe the variations in mortality during the hospital stay, life-table methods were used (Chiang 1968): the patients were divided into three groups, younger than 60 years, 60–69 years and 70 years or older; they were followed until the 27th day, death or day of discharge, whichever occurred first. The quantities estimated are the probability of surviving a specific number of days and the conditional probability of dying on a specific day.

Results

The mean age for the total series was 65.5 years, that for the survivors was 64.2 years and that for the deceased 70.5 years. Table 1 shows the age distribution in decades for survivors and deceased during the CCU period, during after-care and for the total hospital stay. It will be seen that there are highly significant differences between the two groups. Mortality being lower in the three younger decades (40–69 years) and higher in the two oldest (70–89 years).

No significant differences were found for the variables studied between patients dying in the CCU and during after-care. This is exemplified by the results for the age factor in Table 1. This approach will therefore not be commented upon in the following.

Cumulative survival rates during the first four weeks are shown in Fig. 1 for the three age-groups: 59 years or younger, 60–69 years and 70 years or older. It will be seen that the curves decline sharply during the first two-three days and then fall gradually to a cumulative survival rate after four weeks of 85 per cent for the youngest age-groupe, 78 per cent for patients aged 60–69 years and 59 per cent for the oldest age-group. Figure 2 shows the risk of dying on a specific day

TABLE 1. Age distribution among survivors and deceased in CCU, after-care and for the total hospital period.

Age	Total	CCU		After-care		Total-hospital			P
		Survivors	Deceased	Survivors	Deceased	Survivors	Deceased	Mortality in age class	
All patients	2008	1685	323	1473	212	1473	535	26.6%	
	%	%	%	%	%	%	%	%	
<39	0.4	1		1	–	1	–	10	
40–49	6.8	7	3	8	2	8	3	11	
50–59	20.2	22	11	23	11	23	11	15	
60–69	36.3	38	30	39	29	39	30	22	<0.001
70–79	28.1	25	41	23	42	23	41	39	
80–89	7.9	7	14	6	15	6	14	48	
>99	0.3	–	1	–	1	–	1	71	

for the first four weeks. During the first day the risk was about 15 per cent for the oldest age-group (70 years and older) and about 5–7 per cent for the other two groups. After the first three days the risk of dying was 1–3 per cent for all three age-groups.

Previous diseases among survivors and deceased are shown in Table 2. There were no or only

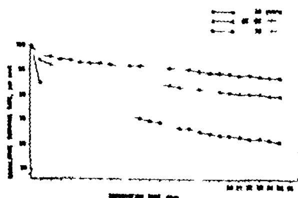


FIGURE 1. Cumulative survival rate during hospital stay for three different age groups.

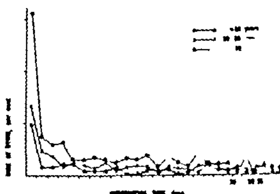


FIGURE 2. The risk of dying during hospital stay for different age groups.

TABLE 2. Previous diseases among survivors and deceased.

Previous diseases		Total	Survivors	Deceased	Mortality in category	P
	All patients	2008	1473	535	26.6%	
Angina pectoris		%	%	%	%	
>6 months		26	25	30	30	NS)
1–6 months		9	9	9	27	
<1 month		22	23	19	25	
No history		38	39	35	25	
No data		5	4	6	31	
Previous infarction						
Two or more		8	8	8	27	NS)
One		26	25	29	30	
None		63	65	59	25	
No data		3	2	4	40	
Congestive heart failure						
Yes		24	21	33	36	<0.001
No		73	77	61	22	
No data		3	2	5	50	
Hypertension						
Yes		25	25	26	27	NS
No		70	72	67	25	
No data		5	3	7	47	
Diabetes mellitus						
Yes		12	11	16	36	<0.001
No		86	88	81	5	
No data		2	1	3	49	

) Might be altered if true data in the "no data" category have an extremely unfavorable distribution.

probably significant differences between the two groups concerning previous diseases, with the exception of CHF and diabetes mellitus, for which highly significant differences were found, the prevalence being higher for the deceased than for the survivors in both cases.

There were no significant differences between survivors and non-survivors concerning the delay time.

Symptoms at onset among survivors and deceased are shown in Table 3. Highly significant differences between the two groups were noted for pain lasting more than 30 minutes, for dyspnoea and for dizziness and syncope with lower frequencies for long-lasting chest pain among the deaths but higher figures in this group for dyspnoea and for dizziness and syncope.

Table 4 shows physical findings – as conscious disturbances, LHF frank pulmonary oedema, hypotension and shock – during the first day among survivors and deceased. The frequencies are significantly higher among the deaths for all these signs ($p<0.001$).

The mean and standard deviation for minimum systolic blood pressure (SBP min) during the first day was for survivors 120±25 mm Hg, and for deceased 104±31 mm Hg. The corresponding values for maximum diastolic blood pressure (DBP max) were 98±16 and 93±19 mm Hg. Maximum heart rate (HR max.) was for survivors 99±23 beats per min. and for non-survivors 113±27 beats per min.

Arrhythmias during the first day among survivors and deceased are shown in Table 5. The deceased had significantly higher incidences ($p<0.001$) of most arrhythmias, though SVB and SVEB were equally common in the two groups.

There were no significant differences between survivors and deceased concerning the localization of the myocardial infarction. However the frequency of uncertain ECG changes was significantly higher ($p<0.001$) among the deceased patients than among survivors (34 and 21 per cent, respectively).

Data on SGOT maximum among survivors and deceased are shown in Table 6. The two groups

TABLE 3. Symptoms at onset among survivors and deceased

Symptoms		Total	Survivors	Deceased	Mortality in category	P
	All patients	2008	1473	535	26.6%	
		%	%	%	%	
Pain						
Pain >30 min.		79	81	72	24	<0.001)
Pain <30 min.		6	6	6	27	
Oppression		7	7	8	30	
No pain		5	4	8	42	
No data		3	2	6	52	
Dyspnoea						
Yes		42	37	54	35	<0.001
No		56	62	41	19	
No data		2	1	5	54	
Dizziness and/or syncope						
Yes		18	16	25	36	<0.001
No		79	82	70	24	
No data		3	2	5	48	
Nausea and/or vomiting						
Yes		45	45	43	26	NS
No		52	53	51	26	
No data		3	2	6	54	

*) See foot f Table 2

TABLE 4 Physical findings during the first day among survivors and deceased.

Physical findings		Total	Survivors	Deceased	Mortality in category	P
	All patients	2008	1473	535	26.6%	
		%	%	%	%	
Conscious disturbances						
Yes		15	7	38	66	<0.001
No		74	84	45	16	
No data		11	9	17	40	
Left heart failure						
Pulmonary oedema		4	3	9	54	<0.001
Pulmonary rales		28	23	43	41	
No items		57	66	32	15	
No data		11	8	16	39	
Blood pressure (systolic)						
Shock		8	1	26	88	<0.001
Hypotension		7	6	11	40	
>90 mm Hg		74	84	48	17	
No data		11	9	15	38	

differed highly significantly and SGOT maximum of 60 199 units per ml was less frequent among the deceased whereas they had higher incidences normal as well as very high values (over 300 is per ml) The mean and standard deviation for T OT was for survivors 157±124 units per ml and for deceased 47± 56 units per ml. The corresponding values for SGPT were 59±67 and 140 5 units per ml

Discussion

The CCU and hospital mortality in this patient series is well in line with other studies of patients with AMI treated in a CCU (Brown et al 1963 Julian et al 1964 Goble et al 1966 Killip and Kimball 1967 Sloman et al 1968 Raftery et al. 1969) The higher mean age of deceased patients compared with those who were discharged alive from hospital after AMI has also been noted by several other authors (Master et al 1937 Rosenbaum and Levine 1941 Helander 1949 Wahlberg 1963 Lawrie et al 1967) On the other hand no or only small differences in mean age between survivors and non-survivors have been found by some authors (Mintz and Katz 1947 Julian et al 1964).

A high proportion of the hospital mortality in patients with AMI occurs during the first two days (Brown et al 1963 Julian et al 1964 Lawrie et al. 1967 Bailey and Beaven 1968 Tucker et al. 1973) The present study clearly shows that the risk of dying is considerably higher especially for the oldest patient group during the first two-three days, after which it is about the same for all three age groups. Helmers (1974) too, found that age plays an important role only at the beginning of the hospital stay It is conceivable that the effect of treatment subsequently outweighs the age factor

Controversial opinions are to be found in the literature about the importance of pre-existing heart diseases. In the present study the deceased group had no or only a slightly increased frequency of pre-existing manifestations of IHD, in the form of chronic angina pectoris (more than six months) and previous myocardial infarction. The high frequency of CHF among the deceased patients was to be expected in view of their higher mean-age and the observed association of CHF to age (Part I) and also because of the unfavourable prognostic influence of a previous history of CHF as found by other authors (Bailey and Beaven 1968 Thompson and Sloman 1971 McGuire and

TABLE 5. Arrhythmias during the first day among survivors and deceased.

Arrhythmias		Total	Survivors	Deceased	Mortality in category	P
	All patients	2008	1473	535	26.6%	
		%	%	%	%	
A-V block I						
Yes		7	5	11	45	<0.001
No		93	95	89	25	
No data		<1	<1	<1	–	
A-V block II						
Yes		5	3	9	52	<0.001
No		95	97	91	25	
No data		<1	<1	<1	–	
A-V block III						
Yes		6	4	13	57	<0.001
No		94	96	87	25	
No data		<1	<1	<1	–	
LBBB						
Yes		9	7	17	49	<0.001
No		91	93	83	24	
No data (1 case only)		<1	<1	<1	–	
RBBB						
Yes		5	4	8	43	<0.001
No		95	96	92	26	
No data		<1	<1	<1	–	
SVT						
Yes		24	19	36	40	<0.001
No		76	81	63	22	
No data		<1	<1	<1	–	
VT						
Yes		13	10	21	43	<0.001
No		84	88	75	24	
No data		3	2	4		
SVB						
Yes		10	10	11	28	NS
No		89	89	88	26	
No data		1	1	1		
SVEB						
Yes		24	25	23	25	NS
No		75	75	76	27	
No data		1	–	1	–	
VEB > 5/min.						
Yes		20	19	25	32	<0.001
No		80	81	75	24	
No data			–		–	
AF						
Yes		12	10	18	40	<0.001
No		87	89	80	25	
No data		1	1	2	–	

TABLE 6 Maximum SGOT among survivors and deceased.

SGOT max. units/ml		Total	Survivors	Deceased	Mortality in class	P
	All patients	2008	1473	535	26 6%	
		%	%	%	%	
1 39		8	6	12	43	<0 001
40 59		10	11	7	19	
60 99		18	21	10	15	
100 149		17	19	10	16	
150 199		12	14	7	15	
00 299		15	16	13	22	
≥300		15	13	21	38	
No data		5	–	20	98	

Kroll 1977) Patients with CHF are expected to have a significantly reduced myocardial reserve and another myocardial infarct in this situation must inevitably mean a bad prognosis.

The high frequency of diabetes mellitus among the deceased could perhaps be explained in part by the age factor because this disease is associated with age as was shown in Part I The unfavourable influence of diabetes on the short term prognosis owever been pointed out by several authors and Truelove 1957 White et al. 1960 s et al 1963 Baly and Beaven 1968 ..Guire and Kroll 1972).

The lower occurrence of chest pain among the deceased is probably explained by the age factor as this symptom is negatively correlated to age (see Part I) The high incidence of dyspnoea among the deceased may be explained along similar lines Dyspnoea is, however often a sign of LHF which greatly influences the short-term prognosis (Meltzer 1968 Norris et al 1968 Sjogren 1970 Thompson and Sloman 1971 Helmers 1974).

The occurrence of conscious disturbances, LHF frank pulmonary oedema, hypotension and shock in patients with AMI nearly always implies a serious condition so the high incidence of these serious signs among the deceased patients was to be expected.

The incidence of serious arrhythmias is high among severely ill patients with AMI (Goble et al. 1966 Meltzer and Kitchell 1966 Killip and Kimball 1967 Weil and Shubin 1968) but it is perhaps surprising that nearly all arrhythmias except SVB and SVEB were significantly more common among the deceased.

In the case of ventricular tachycardia, nodal rhythm and sinus tachycardia, Lown and coworkers (1967) found nearly twice the frequency in deceased AMI patients compared to survivors. These findings agree with the present results.

Elevated values of SGOT correlate fairly well to the damage of hearth muscle (Agress and Kim 1960, Kibe and Nilsson 1967) and high values of serum transaminase activity were accordingly more common among deceased patients. They also had a higher frequency of normal transaminase activity probably because many of them died soon after admission before the serum transaminase activity had had time to rise

A relation was thus found between death in AMI and several variables noted before and during the infarct period. One cannot tell from this study whether these relationships were causal either directly or indirectly For those variables which imply extensive myocardial damage it is perhaps reasonable to anticipate a direct causal relationship The results are of great value for other types of predictions made with the help of multivariate statistical methods.

Chapter II

PROGNOSTIC EVALUATION BY MEANS OF AUTOMATIC INTERACTION DETECTOR ANALYSIS

by

Rune Henning, Torbjörn Lundman and Rein Maasing

A common approach in research is to use a number of independent variables – or predictors – in an attempt to explain the variations in a dependent variable. This is frequently done with statistical methods, such as regression or discriminant analysis.

In the investigation reported in this chapter automatic interaction detector (AID) analysis was used to find those predictors which exert the greatest influence on the short term prognosis. This method also allows for the possibility of interaction between the predictors. The problem can be stated as: Is it possible to identify subgroups – defined by a specific combination of factors – in such a way as to significantly improve our ability to predict the survival status.

Statistical methods

The AID-analysis technique has been developed and introduced only recently (Sonquist and Morgan 1964) and since it may not be as well known as e.g. regression analysis, the philosophy behind this method in comparison to regression analysis is outlined briefly below.

The basic principle is to search for relations between the predictors and the dependent variable in a systematic and reproducible way.

There are two major differences between AID-analysis and methods like regression analysis. Firstly in regression analysis parameters are estimated and the quality of a model is tested by fitting it to the sample. The purpose of AID is not to fit a model, but to provide information so that a good model can be set up – hence its logical place is before model testing. Secondly the analysis is done stepwise but unlike regression analysis, where the explanatory power of an additional predictor is measured over the whole sample and weighed with respect to the other predictors, the most important predictor in successively smaller subgroups is searched for.

Technically it is an unsymmetric branching process where at each decision point the question is raised. "Which dichotomization on which predictor maximizes our ability to predict the value of the dependent variable?" If there is a significant difference in the dependent variable between the two resultant subgroups, if they are fairly large and if there is a gain in the explained variance the group is split, otherwise it is considered final. The predictors may be quantitative and/or qualitative and even on nonordinal scales. No assumptions of linearity or additivity are needed. A more detailed description will be found in Sonquist et al. (1971).

Here the mortality during hospital stay was taken as the dependent variable (dichotomous) and the following rules were adapted. The significance level is 5 per cent and the critical t-values are determined according to Gavatin – Avén (1974). The minimum resultant group size is 25 and the gain in explained variance must be at least 0.6 per cent. Observations were made on three occasions on admission to hospital, during the first day in CCU and during the remaining CCU-stay (mean stay about 3 days). The most serious alternative of each factor on each occasion was used. The three sets of data were analysed by the OSIRIS II computer program AID (1971).

Results

It proved possible to identify several different groups of patients, defined by specific factor combinations, for whom the mortality ~ markedly from the overall average hospital lity of 26.6 per cent.

The most significant predictors associated with markedly increased mortality were LHF age and dyspnoea.

When the analysis is confined to factors on admission the most differentiating factors were LHF age and dyspnoea (Fig 3). Classifying the patients into subgroups according to these factors discloses high mortality rates Patients with LHF and older than 70 years had a mortality of 55 per cent compared with 37 per cent for patients with LHF but younger than 70 years. In this younger group of patients, however those with dyspnoea on admission had a mortality of 45 per cent

In the case of observations during the first day the important variables were the same as on admission, with the addition of VT (Fig. 4) The patients with LHF and older than 70 years had a mortality of 53 per cent. In another group of 46 patients, comprising those younger than 70 years who had LHF dyspnoea and VT the mortality was 72 per cent. Another group with high mortality 56 per cent, was characterized by high age and VT but without LHF Mortality was low 1 per cent, for the group of young patients without LHF

The major predictors for the whole CCU stay

All patients
2008
26 6 /
No
1494
20 %
LHF
Yes
514
46 /
<70
1028
14 /
AGE
≥70
466
34 /
<70
252
37 %
AGE
≥
262
55 /
Yes
180
45 %
DYSPNOEA
No
72
18 /

FIGURES 3 A prognostic table on the admission to hospital. The analyzed variables, the number of patients and the mortality in per cent in each group have been indicated. Boxes with double frame are of special interest se text

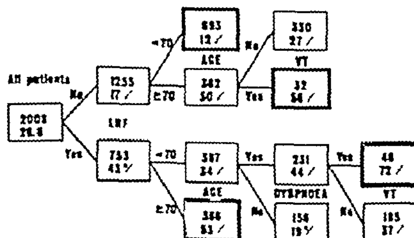


FIGURE 4 A prognostic ta e for the first day in CCU Th ana lyzed variables, numbers of patients and the mortality in per cent in each group have been indicated. Boxes ith dou e frame are of special interest, se text

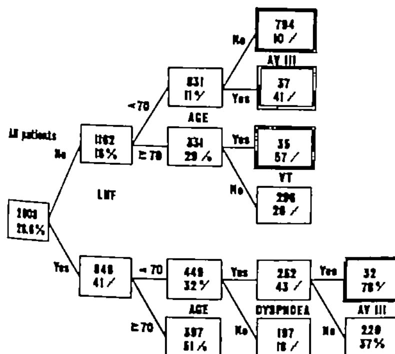


FIGURE 5. A prognostic table for the CCU stay. The analyzed variables, number of patients and the mortality in per cent in each group have been indicated. Boxes with double frame are of special interest, see text

were the same as for the first day in CCU except that A V block III was now a stronger predictor than VT (Fig. 5). Patients with A V block III even if they were young and without LHF had a bad prognosis the mortality in this group being 41 per cent.

Discussion

In the foregoing chapter it was shown that age previous diseases such as CHF and diabetes mellitus, symptoms such as dyspnoea, dizziness and syncope physical findings such as LHF frank pulmonary oedema, hypotension and shock, most arrhythmias and high values of SGOT were significantly more common among the deceased.

The AID-analysis indicates that LHF age dyspnoea, A V block III and VT were the most valuable predictors. All the other variables which were linked with a high mortality in the previous chapter are thus most probably associated with high age and LHF. These other variables contributed no further information and are of less value for the prognosis. Inspection of the choice alternatives at every split point, using observations from the whole CCU stay shows that variables which may provide additional information when studied over the whole sample are such as LBBB, SVT site of infarction and atrial fibrillation.

The young patients without LHF constitute an outstanding group with a very favourable prognosis. However A V block III in this group markedly worsened the prognosis.

In a similar study of 400 AMI-patients, using the AID-analysis Helmers (1974) found that shock, respiratory rate and age proved to be the best predictors. As shock and respiratory rate are both signs of LHF the results of the two studies agree satisfactorily

Chapter III

MORTALITY RISK ESTIMATION BY MULTIVARIATE STATISTICAL ANALYSES

by

Rune Henning, Hans Wedel and Lars Wilhelmsen

INTRODUCTION

It is common experience that various measures of extensive myocardial damage judged from clinical or laboratory observations increase the risk of dying for patients with AMI. In the bedside situation complicated statistical analyses of variables of prognostic importance have not been considered particularly useful. Planning of the treatment in CCU of the duration of hospital stay and particularly planning of intervention trials deserve possibilities to stratify infarct patients according to prognosis. This will be of special importance if early intervention trials aimed at [illegible]ucing the size of the myocardial infarction are be performed. This risk prediction will be more [illegible]itable the earlier it can be set.

By modern computer techniques several methods have become available for this purpose. Models which accept various, linear and non-linear relationships are preferable so that the degree of methodological bias will be reduced by the model used. Computer analyses of such functions are however usually complicated and deserve big computers and long computer time. Such models may also be too complicated to be of clinical usefulness. However a complicated model acceptably fitted to the data and transformable into a system suitable for practical clinical work would be ideal.

The purpose of the present paper was

1 to examine whether certain variables by itself contributed to increased mortality
2 to adapt various mathematical models to predicting the risk of dying during the hospital stay for patients with AMI
3 to investigate the earliest point at which a satisfactory risk prediction could be made
4 to asses the feasibility of constructing a simple clinical prediction system that is sufficiently precise

METHODS

Data collection and choise of variables

The data record contained information about previous diseases, delay time, smoking habits, symptoms at onset, physical findings on admission to hospital and during the following days in CCU, the occurrence of arrhythmias at these times, ECG-findings, various laboratory values and other clinical data. The record also included the duration of stay in CCU and in the hospital, findings on autopsy etc. For every patient up to 179 variables were registered giving about 750 observations during the first seven days, as some variables were observed up to 11 times especially those concerning physical findings and arrhythmias during the CCU stay.

A prognostic index is required at a relatively early stage and as most patients left the CCU after two days, the present estimations have been based on the information available during the first two days. This reduced the total number of observations in the 179 variables to 322. Many of these variables were associated with increased mortality but are certainly inter-correlated.

With the methods used, it is statistically unsuitable to analyse such a large number of variables, especially when inter-correlations have to be adjusted for. They were therefore reduced to 37 with 70 observations, in the light of their importance as assessed from clinical experience from a study of the literature and from the first analysis of this patient series (Part I).

The final selection of 37 variables was divided into four groups

1. preinfarction variables,
2. variables available on admission
3. variables available on the first day
4. variables available on the second day

The variables were coded 1 for a negative observation and 2 for a positive except in the case of those in Table 7 and the following age, SGOT max, SGPT max, minimum systolic blood pressure (SBP min), maximum diastolic blood pressure (DBP max), maximum heart rate (HR max), maximum respiratory rate (RR max) and urine volume, the latter were coded with their absolute value.

The relationship between mortality and a particular variable was evaluated in a bivariate analysis (see statistical methods) without taking other correlated variables into account simultaneously Patients with no value for the variable in question were cancelled. Table 8 gives the levels of significance (at 0.001 0.01 or 0.05) in two-tailed tests for the variables at three different points in time Only associations with $2\,p \leq 0.01$ were considered significant. Twenty-nine variables with 56 observations passed this limit. Of these ECG-localization respiratory rate and urine volume were excluded in the following analyses because they had a high proportion of missing data (up to about 50 per cent). The remaining 26 variables were accepted as significant in the bivariate analysis. At each level of significance the variables have been listed as they occur on the data record, which means that the order within an interval is of no importance

Some variables were excluded from the multivariate analysis because they were strongly associated with other variables. The variables retained were those with the strongest relationship with mortality For this reason the objectively observed degree of consciousness was preferred to a history of mental confusion. Similarly information about smoking was preferred to the duration of this habit, A V block III to A V block I and II, and the frequency of ventricular ectopic beats (VEB) to VEB-type

The question of whether some variables made a direct contribution to mortality was investigated by means ... linear regression analyses (see statistical methods), starting with the preinfarction variables. Some of these were then included in a new stepwise regression together with variables available on admission to hospital. Variables from the first day were analysed in the same way together with some from the time of admission if the latter constituted a more serious observation than on the first day Finally the analysis of variables available on the second day was carried out, again using the most serious observation from the three occasions.

Mortality was estimated with a multiple logistic function and an isotonic model (see statistical methods). In the first analysis the probability of dying was estimated for each patient, using significant variables ($2\,p \leq 0.01$) from the multivariate linear analysis on the three occasions on admission to hospital on the first and on the second day The patient series was than divided into 10 per cent (decile) classes in terms of increasing estimated mortality

In the isotonic model the variables age degree of consciousness and hypotension available on the first day were used in the estimation of mortality To estimate the risk of dying variables available on the second day were used but now hypotension was replaced by SGOT

Statistical methods

The purpose was to examine whether a variable contributed on its own to increased mortality when allowance had been made for the effect on mortality of other correlated variables. This method is referred to as multivariate analysis. As a first step, referred to as bivariate analysis, the question was whether a variable had any association with mortality when no simultaneous allowance was made for other variables. P-values less than 0.01 in a two-tailed test ($2\,p \leq 0.01$) were considered significant, unless otherwise specified.

1 The bivariate analysis. In the bivariate analysis the association was studied between one variable at a time and mortality (y) A permutation test was used to analyse the dependence between x_k and y and the test variable $\sum_{i}^{N} y_i x_{ki}$, where x_{ki} is the observation on variable k. As y_i only has the value 1 if the patient died and 0 if the patient

TABLE 7 Point sc le for ariables gi n other alues than 1 for a negative observation nd for a positive finding

Variables	Definition	Values
Sex	Male	1
	Female	2
Dyspnoe	No dyspnoea	1
	out of breat	2
	rattles in chest	3
Pain	N pain	1
	opression < 30 min.	2
	opression > 30 min.	3
	pain without radiation, < 30 min.	4
	pain without radiation, > 30 min.	5
	pain with radiation, < 30 min.	6
	pain with radiation, > 30 min.	7
Di turbances of consciousnes	No	1
	feeling of fainting	2
	fainting	3
Degree of consciousnes	consc iou	1
	impaired sensorium	2
	nconsciousness	3
LHF	X y neg. phys. neg.	1
	N X-ray phys. neg.	2
	No X-ray phys. pos.	3
	X y neg. phys. pos.	4
	X-ray pos. phys. neg.	5
	X-ray pos. phys. pos.	6
	Frank pulm oedema	7
Hypotension	N	1
	SBP less than 90 mm Hg	2
	Shock	3
VEB-freq ency	No VEB	1
	occasional	2
	1 5 per min	3
	> 5 per min	4
VEB type	N VEB	1
	monofocal	2
	multifocal	3
	paired	4
	oupled (R on T)	5
ECG-localiz tion	N infarction	1
	anterior	2
	lateral	3
	inferior	4
	an lat	5
	ant inf	6
	lat inf	7
	ant lat inf	8
	ncertain	9

[illegible] Significance levels for 37 variables unadjusted for other correlated [illegible]

Before [illegible]	admission	1 day	day	p <
[illegible]	Dyspnoea	Consc.dist.	Cons. dist	
[illegible]	Consc.dist.	LHF	LHF	
[illegible]	Consciousness	Hypotension	Hypotension	
[illegible]	LHF	A–V block I	A–V block I	
[illegible]	Hypotension	A–V block II	A–V block III	
	A–V block II	A–V block III	LBBB	
	LBBB	LBBB	SVT	
	SVT	SVT	AF	
	AF	AF	VEB freq	
	VEB freq	VEB freq	VEB type	
	VEB type	VT	VT	
	VT	SBP min	SBP min	
		DBP max	DBP max	
		HR max	HR max	
		Urine volume	Urine volume	
		RR max	RR max	
			SGOT max	
			SGPT max	
				0.001
[illegible]	Pain		ECG	
	A–V block I			
	A–V block III			
				0.01
		VEB type	WBC	
		ECG		
				0.05
Angina pect.	SVB	SVB	A–V block II	
[illegible] infarction	ECG		Temp	
[illegible]			SVB	
[illegible]			SR max	
[illegible] consumption				

[illegible] variables but have been excluded, see page 15

[illegible], this test of association turns into Fisher's permutation test (Odén and Wedel [illegible]). This test serves to detect any trend in mortality with increasing x_k, that is to say whether the distribution of x_k differs between the persons who died and those who survived [illegible] precisely whether the two distributions are stochastically ordered.

The power of the test has been discussed by Odén and Wedel (1975). The test is powerful against monotone trends in death intensity in x_k but is not suitable if the death intensity is strongly U-shaped in x_k. In this investigation, covering more than 2,000 patients, the test gives practically the same result as the ordinary t test.

II The multivariate analysis. A multiple linear regression model was used to study which variables really contributed to increased mortality (y). In this model it is assumed that $y = \alpha_0 + \sum \alpha_i x_i$ (Hughes et al 1963, Norris et al 1969, Coronary Drug Project 1972). A stepwise linear regression program according to the program library OSIRIS III was used which is easy to handle. The assumption of linearity is apriori not strictly fulfilled. A poor approximation to a linear function can violate the results. The results from the linear regression model could be compared with those of a non-parametric partial correlation analysis, which does not have these restrictions. In this analysis subgroups of patients were formed for every age decade and value of correlated variables and the association between a

TABLE 7 Point sc le for variables gi n othe alues than 1 for neg tive observation nd for a positi find ng

Varia les	Definition	Values
Sex	Male	1
	Fema	2
Dyspnoea	No dyspnoea	1
	out of bre t	2
	r ttles in chest	3
Pain	N p in	1
	oppression <30 min	2
	oppression >30 min	3
	pain without radiation, <30 min	4
	pain ithout radiation, >30 min	5
	pain th radiation, <30 min	6
	p in with radiation, >30 min	7
Disturb nces	N	1
of consciousness	feeling of fainting	2
	fainting	3
Degree of	consciou	1
consciousness	impaired sensorium	2
	unconsciousness	3
LHI	X-ray neg phys neg	1
	N X-ray phys neg	2
	N X-ray phy pos	3
	X-ray neg, phys pos	4
	X-ray pos, phy neg	5
	X-ray pos, phys pos	6
	I ark pulm oedema	7
	N	1
	SBP less than 90 mm Hg	2
	Shock	3
VEB-frequency	N VEB	1
	oc asional	2
	1 5 per min	3
	>5 per min	4
VEB type	No VEB	1
	monofocal	2
	m ltifocal	3
	p ired	4
	oupled (R on T)	5
ECG-localis tion	N infarction	1
	anterior	2
	lateral	3
	inferior	4
	an lat	5
	ant inf	6
	lat inf	7
	ant lat nf	8
	uncertain	9

TABLE 9 Correlation coefficients for 21 variables.

	1	2	3	4	5	6	7	8	9
1. Age									
2. Sex	0.28								
3 Pain	–0.12	0.00							
4 Dyspnoea	0.18	0.08	–0.15						
5 CHF	0.32	0.18	–0.13	0.21					
6. Diabetes	0.12	0.12	–0.06	0.10	0.16				
7 Smoking	–0.42	–0.40	0.08	–0.07	–0.21	–0.13			
8. Consc.dist.	0.16	0.05	–0.10	0.16	0.17	0.03	–0.10		
9 LHF	0.21	0.09	–0.09	0.34	0.25	0.05	0.05	0.25	
10. Hypotension	0.06	–0.02	–0.09	0.10	0.05	–0.03	–0.01	0.56	0.20
11 A–V block III	0.03	0.02	–0.07	–0.02	0.01	0.03	–0.02	0.21	0.03
12. LBBB	0.14	0.04	–0.09	0.17	0.21	0.10	–0.07	0.15	0.20
13. SVT	0.04	0.03	–0.03	0.18	0.05	0.04	–0.01	0.21	0.32
14. AF	0.21	0.07	–0.06	0.10	0.24	0.07	–0.13	0.12	0.10
15 VEB (freq.)	0.02	–0.02	–0.00	–0.03	0.09	–0.00	0.02	0.11	0.14
16 VT	0.00	0.02	–0.04	0.02	0.06	0.01	–0.02	0.20	0.13
17 SGOT max	–0.03	–0.08	0.01	0.04	–0.02	–0.05	0.03	0.07	0.10
18. SGPT max	0.06	0.00	–0.09	0.12	0.10	0.03	–0.04	0.14	0.12
19 SBP min	0.05	0.08	0.01	–0.02	0.03	0.09	–0.10	–0.27	–0.11
20. DBP max	–0.11	0.01	0.09	–0.00	–0.03	0.03	0.04	–0.06	0.10
21 HR	0.14	0.08	–0.08	0.23	0.13	0.07	–0.10	0.24	0.31

	10	11	12	13	14	15	16	17	18	19	20
11	0.21										
12	0.13	0.05									
13	0.17	0.03	0.10								
14	0.05	0.02	0.16	0.02							
15	0.10	0.03	0.08	0.06	0.08						
16	0.22	0.12	0.09	0.09	0.00	0.27					
17	0.16	0.03	0.03	0.07	0.03	0.11	0.03				
18	0.20	0.06	0.08	0.04	0.10	0.03	–0.01	0.67			
19	–0.55	–0.17	–0.08	–0.11	–0.00	–0.10	–0.12	–0.15	–0.15		
20	–0.18	–0.07	–0.02	0.07	–0.01	0.06	0.06	0.00	–0.07	0.42	
21	0.21	–0.02	0.15	0.43	0.21	0.13	0.16	0.11	0.12	–0.13	0.12

Sex and smoking did not add to the information besides age. This applied even when CHF was excluded from the analysis.

Admission variables. Of the preinfarction variables, age and CHF were included in the following stepwise linear regression analysis, which was concerned with variables available on admission. It was then found that each of the factors, age, degree of consciousness, hypotension and shock, SVT and LBBB significantly contributed to increased mortality. None of the other variables available on admission was of any importance for risk prediction.

First day variables. The analysis of variables available on the first day after admission to CCU included the above mentioned five significant variables in addition to CHF and age. LHF, AF and A V block III present on admission were not significantly associated with an increased risk in the linear regression analysis. However, the occurrence of these findings on the first day was of greater importance than on admission (bivariate analysis) and they were therefore included in the linear regression analysis of the first day variables.

HR, LHF and A V block III were now significantl associated with increased mortality in

TABLE 10 Significance levels for I variables adjusted for other correlated variables.

Pre-infarct variables	Variables available on admission	1 day	day	2 p ≤
Age	Age Consciousness Hypotension	Age Consciousness Hypotension HR min	Age Consciousness Hypotension SGOT max SGPT max SBP min	0.001
CHI	SVT LBBB	LHF A V block III SBP min	LHF HR max	0.01
Diabetes				0.05
			A V block III	0.10
		AF SVT		0.20
Sex Smoking	Dyspnoea Pain CHF LHF A V block III AF VPB freq VT	LBBB DBP max		

age degree of consciousness, hypotension and SBP (min) Other variables available on the first day did not yield any information beyound the seven mentioned above It should be noted that the first three variables – age degree of consciousness, hypotension and shock – gave the most valuable information about the risk of mortality inclusion of the other significant variables added relatively little information

Second day variables The analysis of variables available on the second day included SGOT and SGPT and significant variables (2 p≤0.01) from the foregoing analysis As will be seen from Table 10 (column 4) age degree of consciousness, hypotension and shock, SGOT (max) SGPT (max) SBP (min) (2 p ≤ 0.001) LHF and HR (2 p ≤ 0.01) were significant but not A V block III

Estimation of mortality

Multivariate logistic analysis

To estimate mortality only significant variables (2 p ≤ 0.01) from the multivariate linear analysis were used from three different points in time Age degree of consciousness, hypotension and shock were included on each of the three occasions. In addition, the following variables were analysed

1 On admission SVT and LBBB
2 On the first day HR max LHF A–V block III and SBP (min)
3 On the second day SGOT (max), SGPT (max) SBP (min) LHF and HR (max)

The estimates of the parameters β_i in the logistic model are given in Table 11 With these coeffi-

cents given it is possible to obtain an estimate of the mortality for each patient. An example to illustrate this is given below Table 11

TABLE 11 Constants in the logistic function.

Variable X_i	Constant β_i on: admission	1 day	2 day
Age	0 056	0 055	0 059
Consciousness	0 687	0.741	0 451
LHF	–	0 081	0 100
Hypotension	0 910	0 647	0 745
A V block III	–	0 614	–
LBBB	0.403	–	–
SVT	0.245	–	–
SBP min	–	–0.0087	–0 0111
HR max	–	0.0131	0 0043
SGOT max	–	–	0 0018
SGPT max	–	–	0 0023
Constant 3	–7.535	–8.103	–7.209

1 $\lambda \quad \beta_1 \; X_1 \quad \beta_2 \; X_2 + \beta_3 \; X_3 \qquad + \beta_n \; X_n \quad \beta_0$

2 $p(X_1 \ldots X_n) \quad \dfrac{\lambda}{1 \quad \lambda}$

The probability of dying e.g. mortality (p) has been estimated for every patient on three points in time according to formula 1 and 2. The following example illustrates this:

A patient 65 years old (X_1 65), conscious (X_2 1), frank pulmonary oedema (X_3 7), no hypotension (X_4 1), no A–V block III (X_5 1). Minimum systolic blood pressure 110 mm Hg (X_8 110) and maximum heart rate during the first day was 170 beats/min (X_9 170). The estimated probability of dying will be according to formula 1 and 2 for the first day

λ 0.055 65 0.741 1 0 081 7 0 647 1 0 614 1 – 0.0087 110 0 0131 170 – 8.103

λ – 0 69 which gives

p 0.33 according to formula 2
(or Geigy 1970 p 56)

The result of the logistic analysis with estimated mortality divided into decile classes from the three occasions, is shown in Table 12 together with the observed mortality. The observed and estimated mortality are low in the first four-five classes, and increase by about 2–5 per cent between each of the first eight decile classes. However, in the two highest classes the increase is considerably greater and amounts to as much as 30 per cent between the two last classes. The observed mortality was as high as about 70 per cent in the highest decile class.

Isotonic model

Using an isotonic model mortality was estimated from the three variables age degree of consciousness hypotension and shock on the first day The results are presented in Table 13 which shows the estimated mortality in different groups. The Table also shows the number of patients (expressed per 1000) in the different groups. It will be seen that high-risk groups consisted of unconscious patients with or without hypotension and shock, with an estimated mortality of 76–94 per cent here the age factor seemed to be of little importance Low-risk groups consisted of conscious patients without hypotension in younger age groups (up to 69 years) the estimated mortality was calculated to 4–13 per cent rising for the two oldest groups (70 years and above) to 21 and 32 per cent respectively With the isotonic method the observed mortality for the first day in the highest and lowest 10 per cent classes were found to be 79 and 5 per cent, respectively Compared with observed mortality in the highest and lowest decile classes found in the logistic method (76 and 2 per cent, respectively) there was a good accordance.

The three variables age degree of consciousness and SGOT available on the second day were used to estimate the mortality The results are presented in Table 14 Comparing the two tables (Table 13 and 14) similarly results will be found. High-risk groups consisted mainly of unconscious patients. Low risk groups consisted of younger conscious patients. SGOT did not contribute much to risk prediction besides age and degree of consciousness.

In Figure 6 distribution of deceased in the decile classes of estimated mortality is calculated for the first day More than 50 per cent of the deceased are found in the two highest deciles for both methods.

Evaluation of Peel's index

Peel et al (1962) have constructed a prognostic index from the following variables: age sex previous history of cardiovascular diseases,

TABLF 1.. O served morta y and estimated risk in decile classes at thre different points in time

	Decile cla										
	1		3	4	5	6	7	8	9	10	T tal
I On admission											
100 x observed mortality	4 6	9 6	10 5	16.0	19.6	3 7	23 7	32.0	44 7	68 5	5 2
100 x estim ted mor lity	6 9	10 3	13 2	15 2	18 3	21.0	4 9	30 5	40 3	70 4	
II On th first day											
100 x observed mortal ty	1	4 1	10 8	9 7	1..3	17 9	4 1	26	40 5	73 9	2 3
100 x estimated mortal ty	4 1	6 4	8 3	10.1	12 6	15 9	20 2	7.5	42.3	73 4	
III O the second day											
100 x observed mortality	2 1	2 6	5 8	6 9	1	13 2	14 8	25 4	41.2	68 3	19 3
100 x estimated mortality	3 3	5 2	7 0	8 4	10 6	12 9	17 2	3.5	35 0	67 7	

Each decile class consist of 10 per cent f the number of patients ordered after the estimated mortality according t the formula in table 11

TAPLE 13 Estimated mortality calculated by the isotonic model based on three observations (age consciousness and hypotension) available on th first day

... tion 1)	Degree of consciousness		Age 49	50 59	60 69	70 79	80
	Unconscious 3	per 1000		5	15	35	7
		%	80	60	84	91	94
Shock	Dist ous 2	per 1000	3	6	15	14	5
		%	60	60	65	76	94
	Cons us 1	pe 1000	3	2	6	4	2
		%	20	20	24	46	94
	Unconscious 3	per 1000	0	1	5	1	4
				76	80	80	80
Hypotension	D t ous	per 1000	0	3	7	5	4
		%		42	47	62	62
	Conscious 1	per 1000	0	17	20	17	5
		%		8	24	44	44
	Uncons ou 3	per 1000	0	2	5	5	5
		%		76	76	76	76
N hypotension	Dist ous	per 1000		10	35	39	14
		%	0	20	4	46	46
	Conscious 1	per 1000	60	150	5	185	41
		%	4	7	13	21	3

See ta le 7

The upper row in every group shows th he number of patients per 1000

Th lower row gives th estimated morta ty in per cent

TABLE 14 Estimated mortality calculated by the isotonic model based on three observations (age consciousness and SGOT max) available on the second day

SGOT Units per ml	Degree of consciousness		Age 49	50–59	60–69	70–79	80
>125	Unconscious	per 1000*	*0*	*1*	*4*	*4*	*2*
	3	%	–	80	80	84	84
	Dist. consc.	per 1000	1	1	8	11	3
	2	%	46	46	46	63	84
	Conscious	per 1000	3	12	19	7	2
	1	%	14	15	33	50	50
76–125	Unconscious	per 1000	0	1	2	3	1
	3	%	–	80	80	84	84
	Dist. consc.	per 1000	2	6	10	8	2
	2	%	38	38	38	50	50
	Conscious	per 1000	8	23	30	33	9
	1	%	10	10	24	26	40
41–75	Unconscious	per 1000	0	3	8	4	3
	3	%	–	72	80	84	84
	Dist. consc.	per 1000	2	5	16	17	6
	2	%	31	31	33	50	50
	Conscious	per 1000	32	63	112	76	16
	1	%	3	6	11	19	39
<40	Unconscious	per 1000	1	4	9	17	7
	3	%	*72*	*72*	*80*	*84*	*84*
	Dist. consc.	per 1000	2	9	25	24	12
	2	%	31	31	33	50	50
	Conscious	per 1000	24	68	113	90	18
	1	%	3	6	1	19	30

* See Table 7
** See Table 13

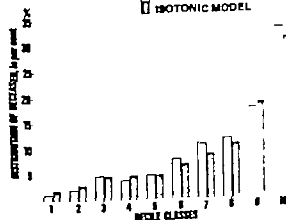


FIGURE 6. The distribution of deceased in decile classes in the logistic and isotonic model.

cardiac failure cardiac rhytm and electrocardiographic changes. This point-scale was applied to the present series, which called for slight modifications concerning the ECG-changes. The result is presented in Table 15 in which the distribution of patients (no per 1000) and the mortality (in *per cent) in the score-classes are compared bet*ween the two series.

DISCUSSION

The aim of the present study was to find suitable variables for predicting an increased risk of dying during a hospital stay A major problem concerned the large number of variables. To reduce this number a selection was made and then a division into four groups, followed by a final selection using bivariate analysis. Only the 29 variables wi /

TABLE 15 *Evaluation of P ... Ti and x*

Score	Present series N 2005: N per 1000	Present series N 2005: Mortality (%)	Peel series N 6 8: No per 1000	Peel series N 6 8: Mortality (%)
1 8	195	11 7	3	5
9 1	2	14 5	10	12 5
13 16	307	23 9	25	23 4
17 20	192	40 5	119	53 4
1–25	84	65 8	54	88 5
Total		26 6		20 6

a ? p value less than 0.01 were accepted for analysis by stepwise linear regression.

The correlation coefficients for the association between two variables show that many of them are strongly inter-related However the correlation coefficient does not illustrate all possibilites of association especially well It is also incomplete as a measure and should be used only as a rough estimate of an association

Multivariate analysis

analysing the association between a variable mortality it may happen that this correlation explained in part by other correlated variables In this study an attempt has been made to analyse the contribution of some relevant variables to an increased mortality allowing for other associated variables First an example is given to illustrate the importance of taking correlated factors into account Smoking shows a negative association to mortality suggesting that smokers have a greater probability of surviving the hospital stay than non smokers However Table 9 also shows a negative correlation to age indicating that elderly people smoke less If allowance is made for the age factor in a multivariate analysis it might be found that for a given age there is no association between smoking and mortality

The stepwise linear regression analysis was used for five prein farction variables Of these age was by far the most important and was included in all the following analyses CHF also yielded valuable information by itself even allowing for the strong association with age Diabetes did not appear to give much further information perhaps due in part to the association with age Neither did sex give

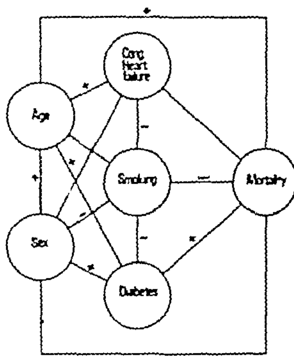


FIGURE 7 An illustration of the association between some variables and mortality

any information notwithstanding the higher mortality among women this too may be explained by the relatively strong association with age

The association between some variables and mortality is illustrated in Fig 7 A positive association is denoted by + and a negative by – where a bivariate association disappeared in the multivariate analysis the sign occurs in brackets.

In the following analysis – of variables available on admission to hospital – age degree of consciousness, hypotension and shock, SVT and LBBB were *important predictors of increased* mortality CHF was now not significant, probably because of the relatively strong association with some of the new significant variables eg LBBB. Neither were dyspnoea and LHF now significant probably because of the relatively strong association with some of the five significant variables mentioned above The reason why some arrhythmias were still of no substantial importance could be their low incidence on admission especially A V block III and VT (see part I)

In the analysis of variables available on the first day HR max was added to the three most important predictors (age degree of consciousness and

hypotension) from the foregoing analysis. On top of these four variables, some further information was provided by LHF A V block III and SBP (min) On the other hand AF SVT LBBB and DBP (max) were of no importance as predictors. As will be seen in Table 9 there was an association between some of these variables and the foregoing seven significant predictors, for instance between age and AF and between SVT and degree of consciousness. This association may help to explain why these variables were not significant in the multivariate analysis.

Analysing the variables available on the second day six predictors were most importance age degree of consciousness and hypotension as before plus SGOT (max) SGPT (max) and SBP (min). Moreover LHF and HR (max) were still of some importance but not A V block III.

The varying importance of complete heart block at the three points in time is interesting. The weak influence of A V block III at admission is probably explained by the low incidence of this arrhythmia at that time only 2 per cent. Its relatively high significance on the first day can perhaps be explained by the complication of complete heart block in anterior myocardial infarction, a combination that is associated with a high mortality (Friedberg et al. 1968 Norris et al. 1969 Lemberg et al. 1971) If the patients survive the initial phase or develop A V block III more gradually as in inferior infarction, the prognosis is better This may perhaps partly explain why the predictive ability of A V block III varies so much between the three points in time

Estimation of mortality

In the multiple logistic analysis tested in this patient series, the observed mortality differed from the estimated risk by about ± 2 per cent in various decile classes. The estimated risk of dying is about 70 times greater in the highest classes than in the lowest decile

The uncertainty of many variables available on admission was perhaps greater than for those available on the first day The difference in the estimated risk between the highest and lowest decile class was greater for the first day than for admission (69.3 and 63.5 per cent, respectively). There was however relatively little difference between the same decile class on these two occasions. It was therefore possible to predict the probability of dying rather reliably even as early as on admission. The evaluation of variables available on the second day did not increase the predictive ability This probably reflects the larger percentage of missing data, which complicates the analysis and reduces the possibility of obtaining a better prediction.

Similar methods were used by Vedin (1974) to predict the mortality during a two-year follow-up of 316 men discharged alive from hospital, and in the Coronary Drug Project (1972) to investigate the prognostic importance of ECG after myocardial infarction during a 3-year follow-up In these studies there was good agreement between the observed and the estimated mortality Close accord was also achieved by Wilhelmsen et al (1973) using a logistic model to estimate the risk of CHD in a healthy population, even when this method was tested in another population.

The isotonic model was able to differentiate between high-risk groups with an estimated mortality of about 76—94 per cent and between low-risk groups with an estimated probability of less than 10 per cent. The influence of the age factor seemed to be relatively weak in the high-risk groups, i.e. unconscious patients with or without hypotension or shock, but stronger in low-risk groups, where the estimated probability of dying increased with age.

The combination of variables used in the isotonic model as is shown in Table 13 is only an example Of course other combinations of variables can be used. Age is a variable with a great predictive ability and it has been used in most combinations. The other variables, hypotension and degree of consciousness can be exchanged by other variables. However these two variables gave valuable information about mortality according to the multivariate analyses why these variables were used firstly This combination had also the best predictive ability in the isotonic model. An example of another combination of variables is given in Table 14 where the predictive ability of the combination of age, SGOT and degree of consciousness is shown.

Laboratory values, as SGOT may be regarded

more valid variables than many clinical observations as hypotension and degree of consciousness. This may or may not be correct However the combination of age SGOT and degree of consciousness did not have a better predictive ability than the combination of age hypotension and degree of consciousness. Thus, analysis of variables available at the bedside and at an early stage had a good predictive ability and as good as laboratory variable gave later This indicates that clinical observations, if collected in a standardized way may be of great value

The theory behind isotonic regression is relatively simple but the algorithm which gives the solution is complicated to use in a study like this, with many variables and a large number of patients. Even with modern computer techniques it is not suitable to use more than three variables in large series like this one However in the worked up form the combination of predictors is easy to survey and consequently this method might be useful in clinical work. The model can perhaps be suitable for creating homogeneous groups which is ... rtant for an adequate assessment of drug

...se results should of course be tested on ... r series of patients with AMI in order to determine whether the function gives good agreement between estimated and observed mortality It is important to realize that for the individual patient the estimated mortality is rather uncertain. However by creating homogeneous groups one can substantially reduce the uncertainty in the estimation of mortality for similar groups.

The comparison between the present series and that of Peel showed good agreement in the case of the two classes with scores of 9–12 and 13–16 the percentage mortalities are almost identical in both cases. The distribution of patients (calculated as no per 1 000) for the two groups combined is also of nearly the same order The mortality in the group with a low score (1–8) is somewhat lower in Peel's series. The distribution of patients to this group is different in the two series which could help to explain the differences in mortality in these groups and perhaps the lower total mortality in Peel's series. However the mortality in Peel's series was higher in the two highest score-classes. It is perhaps remarkable that there is such good agreement between the observed and estimated mortality within the two series despite to the differences in the composition of the patients groups.

To enable a comparison between different methods Peel's patient series (196?) as well as Hughes (1963) and Norris series (1969) were divided into decile classes after estimated mortality according to their own scoring systems. The observed mortality in the highest decile class was for Peel's series 64 per cent, for Hughes 100 per cent and for Norris 78 per cent The overall mortality was 21 per cent in Peel's series 32 per cent in Hughes series and 27 per cent in Norris series. The higher predictive ability in Hughes series might at least partly be explained by the higher overall mortality among these patients.

The hospital mortality for patients with acute myocardial infarction has been persistently high. The advent of coronary care units permitted an improvement, especially in the early phase covered by the CCU stay when the risk of dying is particularly high. In two studies, which compared the hospital mortality for patients with AMI treated in an ordinary medical ward with that for patients admitted to a CCU it was possible to show a 50 per cent reduction of the hospital mortality (Christiansen et al. 1971 Hofvendahl 1971). This benefit was achieved mainly by the early detection and prophylactic or direct treatment of potentially serious arrhythmias. Norris et al. (1969) have shown that the reduction was achieved in patients whose infarctions were of moderate serverity. Even so, the mortality in AMI is still high, especially for patients with severe heart failure, hypotension, cardiogenic shock and secondary arrhythmias.

Treatment in a CCU is expensive and it is not feasible to admit all patients for the duration of their illness. This makes it all the more important to know about the prognosis for patients with AMI. Classification of patients in homogeneous groups improves the chance of shortening the care of patients in low-risk groups and providing more intense treatment for those in high-risk group. It also provides a basis for choosing and assessing a method of treatment more adequately.

The possibility of comparing different patient series and results will also be enhanced by the creation of homogeneous patient groups. A prognostic index and a classification in homogeneous groups has to be relatively easy to apply in clinical work, otherwise they may be of little value in daily routines.

When analysing many variables and their influence on the dependent variable, in this case the hospital mortality associations will be found between many of them. An important purpose was to identify a few variables which together contributed as much as possible to the information about mortality. A combination of these few but highly significant predictors ought to provide increased information about prognosis.

The classification of patients with AMI according to some clinical findings has been used by many authors (Connor and Holt, 1930, Master et al. 1937 Rosenbaum and Levine 1941 Mintz and Katz 1947 Billings et al. 1949 Helander 1949 Björck et al. 1957 Honey and Truelove 1957 Harnagel et al. 1957 Beard et al. 1960, Peel et al. 1962, Wahlberg 1963 Robinson et al. 1964 Lawrie et al. 1968 Sloman et al. 1969). Using computer techniques and various statistical methods more sophisticated systems or prognostic indices have been developed both for the short and/or long-term prognosis (Hughes et al. 1963 Lemlich 1965 Norris et al. 1969 and 1970, Antonini et al. 1970, Bulloch et al. 1970, McHugh and Swan 1971 Coronary Drug Project 1972, Chapman and Gray 1973 Helmers 1974 Vedin 1974).

Controversial opinions are to be found about the importance of many of these variables. As with other diseases many factors contributed to a poor prognosis for patients with AMI. However the association between hospital mortality and some variables as age severe left heart failure hypotension and shock, SGOT and arrhythmias such as total heart block, LBBB and ventricular tachycardia could be found in many investigations.

In the present work the aim was to investigate the influence of various variables on hospital mortality in order to create possibilities of classifying the patients in as homogeneous riskgroups as possible. Different statistical methods as AID-analysis, linear stepwise regression analysis, multiple logistic analysis and an isotonic model were used. Variables, having strong association with hospital mortality as age CHF degree of consciousness, LHF hypotension and shock, HR, RR and arrhythmias as A V block III LBBB SVT VT and blood pressure SGOT and SGPT will be discussed separately. Some variables such as diabetes, previous myocardial infarction and arrhythmias such as AF and VEB were not shown to have any great influence on hospital mortality in this work.

Age has been found to be an important factor

for mortality in patients with AMI (Peel et al 1962, Hughes et al 1963 Lemlich 1965 Norris et al. 1969 Antonini et al. 1970, Bullock et al. 1970) The present series indicates that mortality increased steeply after the age of 65 (Fig 3 Part I) By use of AID-analysis it was shown that age and LHF were the most important predictors of mortality

In the stepwise linear regression analysis, age contributed important information about mortality when three different points on time were investigated viz. on admission on the first day and on the second day When using the isotonic model it was shown that the age factor had a greater influence on mortality among moderately ill patients than among severely ill patients.

The poor prognosis for patients with a previous history of CHD has been pointed out by several authors (Honey and Truelove 1957 Harnapel et al 1959 Bailey a d B aven 1968 Thompson and Sloman 1971 McGuire and Kroll 197?) In the AID ana us this ariable did not appear among the most five important factors Analysing five ... rfar ti n variab es in stepwise linear regres... LHF was, apart from age the most important ... or for mortality However in the following ... regression analysis CHF gave no information of pr... ... ance

Diabetes me itus has been of unfavourable influence on the short term prognosis (Hughes et al 19?3 Ba... d Braven 1968, McGuire and Kroll 19... ... others have not found diabetes associated with higher mortality (Norris et al 19?8 Thompson and Sloman 1971 Helmers 1974) In analysing the preinfarction variables using ... regression analysis diabetes gave some predict... ... on but this was not the case in the f... stepwise regression analysis

Depressed ... consciousness was found to be of importance as a predictor in all the regression analyses and was therefore also included in the isotonic model even though this predictor must be regarded as a ... variable

B... et al (1974) found that the presence of cloudy sensorium made the prognosis significantly worse and Helmers (1974) found that the short term prognosis for patients with disturbances of consciousness was significantly worse compared with patients without these symptoms These findings are in agreement with those in the present study

LHF was found to have a strong predictive ability in all the methods used in this work. In the AID-analysis LHF was the first chosen variable (i.e the strongest predictor) According to the results in this analysis patients with LHF had a mortality more than twice that of patients without LHF In the multivariate analyses LHF yielded valuable information even if this variable did not reach the significance level of 0.001 probably owing to its association with other important predictors such as age hypotension and heart rate The poor prognosis for AMI patients with LHF has been pointed out by many authors (Peel et al 196? Hughes et al. 1963 Lemlich 1965 Stock 1967 Norris et al 1968 Chapman 1970 Sjogren 1970 Hofvendahl 1971 Helmers 1974) These findings were confirmed by those in the present work However LHF did not appear among the four most important predictors and was not used in the isotonic model. Hypotension and shock proved to be one of the three most important predictors for mortality and was included in the stepwise linear regression analysis on three different points in time as well as in the logistic and isotonic models In the later analysis it was found that high-risk groups consisted of unconscious patients with hypotension or shock with an estimated mortality of 75–90 per cent, even in younger age groups. Age seemed to have very little influence on mortality in these seriously ill patients

The unfavourable prognostic importance of hypotension and shock has been found in many studies (Peel et al 1963 Hughes et al 1963 Braunwald 1967 Shubin and Weil 1967 Bloomfield 1970 Ban et al 1971 Nyquist 197?) The establishment of CCUs has not decreased the mortality among these severely ill patients (Norris et al 1969 Bloomfield 1970 Scheidt et al 1970) This is also confirmed by the results in the present study

The incidence of most arrhythmias was found to be higher among deceased patients (Table 5) Arrhythmias which contributed valuable information about mortality were A-V block III LBBB SVT and VT In the analysis of variables on the first day by the AID-method VT was found to be

a predictor but analysing variables for the whole CCU-stay A V block III was now shown to have greater predictive ability than VT Even young patients with A V block III but without signs of LHF had a poor prognosis. In the stepwise linear regression analyses A V block III was of varying predictive ability with greater influence on the first day compared with that on admission to hospital and on the second day

High mortality for AMI patients with complete heart block has been found in many studies (Friedberg et al. 1968 Brown et al. 1969 Blomfield 1970, Mogensen 1970 Chapman 1971 Sloman et al 1971) and is well in line with the present findings. LBBB and SVT had some predictive ability in the stepwise linear regression analysis ($2p < 0.01$) but not in the following analyses, probably due to the close association with other variables (age LHF Hypotension and HR).

When SGOT and SGPT were included in the analysis of variables available on the second day each of these laboratory values gave valuable information of mortality in spite of their close interrelationship However in the isotonic model, the use of a combination of SGOT instead of hypotension and shock did not provide a better predictive ability

Several authors have found a near association between SGOT and hospital mortality (Hansen and Laursen 1957 Bruce et al. 1958 Keele et al. 1958, Agress and Kim 1960 Kibe and Nilsson 1967) and a significantly increased hospital mortality rate with high values of SGOT has been reported (Stock 1967 Isacsson et al. 1969 Chapman 1971) Chapman and Grey (1973) used SGOT as one of three significant independent variables (in addition to cardiogenic shock and oliguria) according to multiple regression analysis in their prognostic index for patients with AMI treated in a CCU Helmers (1974) found a correlation between maximum SGOT and prognosis in patients surviving the first day

Significant variables in the bivariate analysis were analysed in stepwise linear regression. Some variables were shown to give very little further information besides twelve significant variables ($2p < 0.01$) probably because inter-correlations. However the significant variables were also inter correlated but in spite of this fact contributed on its own to valuable information as predictors.

Besides the age factor most of the other variables which contributed to increased mortality risk can be regarded more or less as indices of the size of the acute myocardial infarction. Patient, who have more than one of these twelve significant variables simultaneously formed a group with very high risk and ought probable to be supervised more intensively and treated longer in hospital.

On the contrary there is also possibilities to find low-risk groups with a low mortality risk, of 4–8 per cent. They consisted of as a rule young patients without signs of severe infarction. These patients need probably not be monitored any longer period, can be mobilized early and might perhaps soon be discharged from the hospital when the acute phase of the illness is over As can be seen from fig. 4 and 5 these low risk-groups consisted of more than a third of the total number of patients.

The present work was a co-operative study and the patient series was collected from twelve Swedish hospitals. It may be regarded as an advantage to collect series from several hospitals in order to get a representative series of general characteristic. When considering the generalization on the results in the present work, it should be born in mind, that all these patients were treated in a coronary care unit. This might perhaps have influenced the results in such a way that the importance of arrhythmias as predictors have been less than otherwise should have been the case. Especially for patients, whose infarction was of moderate severity the importance of arrhythmias might have been greater without these possibilities of early detection and prophylactical or direct treatment of potentially serious arrhythmias.

The variables used in the present study were collected in a manner used in the daily clinical work without advanced methods or techniques. In spite of this, good predictive ability was achieved with bedside observations that are available at the early stage of acute myocardial infarction.

The patient series was collected during 1969 from twelve Swedish hospitals with CCUs and consisted of 7008 patients with acute myocardial infarction

The aim of this Part was to investigate the short term prognosis for patients with AMI by using different statistical methods for determining factors with the greatest importance for mortality during hospital stay

Chapter I A group comparison was made between survivors and patients who died during the hospital stay A relation was found between mortality and several variables noted before and during the infarct period.

Chapter II Automatic interaction detector (AID) analysis was used to find those predictors which exert the greatest influence on the short term prognosis at three different points in time

1 Analyzing variables available on admission the st differentiating factors were left heart re age and dyspnoea
2 e analysis of variables available during the first day in CCU showed that important variables were the same as on admission with addition of ventricular tachycardia
3 The major predictors for the whole CCU stay were the same as for the first day in CCU except that A V block III was now a stronger predictor than VT Even young patients with A V block III but without LHF had a poor prognosis

Chapter III The purpose was

1 to examine whether certain variables by itself contributed to increased mortality
2 to adapt various mathematical models to predicting the risk of dying d ring the hospital stay for patients with AMI
3 to investigate the earliest point at which a satisfactory risk prediction ould be made
4 to asses the feasibility of constru ting a simple clinical prediction system that is sufficiently precise

The relationship between mortality and a certain variable was evaluated in bivariate analysis without taking other correlated variables into account simultaneously Only significant associations were considered (*p < 0.01) Multivariate analysis was used to study significant variables in the bivariate analysis to investigate which variables really contributed to increased mortality by means of stepwise linear regression

1 Preinfarction variables Age and congestive heart failure were strongly associated with hospital mortality
2 Admission variables Besides age degree of consciousness, hypotension and shock, supraventricular tachycardia (SVT) and left bundle branch block (LBBB) significantly contributed to increased mortality
3 First day variables Age degree of consciousness, hypotension and shock, HR max minimum systolic blood pressure (SBP min) LHF and A V block III were now significantly associated with mortality
4 Second day variables Age degree of consciousness hypotension and shock, SGOT (max), SGPT (max) SBP (min) LHF and heart rate (HR max) were now significant but not A V block III

To estimate mortality a logistic function and an isotonic model were used The estimated mortality was in the logistic analysis divided into decile classes from three occasions. The observed and estimated mortality were low in the first five classes but increased considerably in the two highest classes and was about 70 per cent in the highest decile class.

In the isotonic model the mortality was estimated from the three variables age degree of consciousness, and hypotension and shock on the first day High-risk groups consisted of unconscious patients with or without hypotension and shock with an estimated mortality of 76 94 per cent The age factor seemed to be of little importance in these high-risk groups. Low risk groups consisted of onscious patients without hypotension In younger age groups (up to 69 years) the estimated mortality was calculated to 4 13 per cent

The three variables age degree of consciousness and SGOT available on the second day were also used to estimatic the mortality Similarly results were found for these two combinations. SGOT did not contribute much to risk prediction besides age and degree of consciousness. This combination of variables had not a better predictive ability than the combination of age degree of consciousness, hypotension and shock. Analysis of variables available at bedside and at the early stage had a good predictive ability and as good as laboratory variables gave later

The worked-up form of the combinations according to the isotonic model is easy to survey and this method can perhaps be useful in clinical work.

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Experimental and Clinical Aspects on Preservation of the Ischemic Myocardium

Edited by Åke Hjalmarson and Lars Werkö

A. Lindgren & Söner AB Mölndal, Sweden, 1976

The development of myocardial ischemia and infarction is a dynamic process due to an imbalance between myocardial demand and coronary artery supply of fluid, oxygen and substrates. This process could hopefully be modified positively by various selective measures instituted as early as possible after or even better before onset of severe chest pain. The clinical therapy for preservation of the jeopardized myocardium ought to be based on knowledge of the pathophysiological mechanisms behind the development of acute myocardial ischemia. During the last few years there has been an increasing interest in factors of importance for the development of an acute experimental myocardial infarction. Based upon the results from these experimental studies attempts have been made to preserve the ischemic myocardium in patients with acute myocardial infarction in order to find a clinical therapy that could modify the infarct size in man. One main problem in such clinical studies has been to find a method for estimation of the infarct size in man. Surface mapping of ST-segment elevation, serial measurements of CPK, and the gamma camera technique have been suggested for estimation of infarct size but all have their limitations and none has proved to be accurate.

The purpose of the present symposium, held in Copenhagen in June 1975 was to present and discuss "Experimental and Clinical Aspects on Preservation of the Ischemic Myocardium". The attempt was made to interchange ideas and opinions between appropriate experts working on the biochemical level of the myocardial infarction and those studying its clinical problems. The symposium was made possible through a generous grant from the ICI Pharmaceuticals Division, which is highly appreciated. We are also indebted to Mr Per-Erik Hörnkvist of ICI Pharma AB who was responsible for all technical arrangements of the symposium and to Miss Eva Claus and Miss Anita Gunnarsson for their skilful typing of all discussions and correcting of manuscripts and proofs

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CONTENTS

EXPERIMENTAL ASPECTS ON PRESERVATION OF THE ISCHEMIC MYOCARDIUM

CLINICAL ASPECTS ON PRESERVATION OF THE ISCHEMIC MYOCARDIUM

RATE LIMITING STEPS OF CARBOHYDRATE AND FATTY ACID METABOLISM IN ISCHEMIC HEARTS

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SUMMARY

Control of glycolysis and fatty acid oxidation in ischemic myocardium was studied in isolated working rat hearts. Coronary flow was reduced to the *whole heart*. In ischemic tissue, oxygen consumption, glycolysis and fatty acid oxidation all decreased in proportion to the restriction in coronary flow. Inhibition of glycolysis developed at the level of glyceraldehyde 3-phosphate dehydrogenase. Restricted flux through this step appeared to result from accumulation of lactate, H and NADH. The rate of glycolysis was inversely related to accumulation of lactate. Additions of high levels of lactate to the perfusate inhibited glycolysis in aerobic, anoxic and ischemic hearts. *The mechanism of this effect of lactate in anaerobic hearts is unknown, but does not appear to be related to pH changes.*

Oxidation of fatty acids was restricted at the level of β-oxidation and high levels of both long-chain acyl CoA and carnitine derivatives accumulated.

INTRODUCTION

The concept that ischemic myocardium may be protected from irreversible damage has in recent years received considerable attention. Occlusion of a coronary artery is known to result in a heterogenous pattern of blood flow in and around the area of involved tissue (1). Blood flow ranges from about 5 % *of normal near the center of the ischemic zone* to normal in the peripheral areas. Attempts to protect the ischemic tissue have included improved coronary blood flow through use of vasodilators and increased diastolic aortic perfusion pressures, decreased contractility to bring energy utilization into better balance with the decreased energy supply and metabolic alterations designed to improve anaerobic production of ATP. The purpose of the present study was to determine the rates of production and sources of ATP that might be expected in the various areas of tissue in relation to the amount of coronary flow received. In addition, regulation of *the ATP producing metabolic pathways* has been investigated at several rates of flow. For these studies, a model of ischemia was used in which coronary flow was reduced to the whole heart. This allowed the rates of coronary flow and of substrate utilization to be determined with more precision than can be achieved in a model of regional ischemia.

In previous studies, a 60 % reduction in total coronary flow resulted in about a 30 % reduction in oxygen consumption, a 70 % reduction in fatty acid oxidation and a *two-fold stimulation of glycolysis* (2, 3). A 90 % reduction in flow produced a larger percent decrease in oxygen consumption and fatty acid oxidation and inhibited glycolysis. Total ATP production was restricted by 33 and 85 percent at the two flow rates, respectively.

METHODS

Whole heart ischemia was induced in isolated working rat hearts by placing a one way valve in the aortic outflow tract (4). This valve prevented retrograde perfusion of the coronary arteries during diastole and reduced coronary flow by 60 % initially. Ventricular failure followed the reduction in flow by 5-10 min. In hearts that were not electrically paced, failure was expressed as a reduction in heart rate and an increase in the end diastolic ventricular *pressure but with no decrease in peak pressure development.* In this case, coronary flow was maintained at about 40 percent of control rate (2). In electrically paced hearts, failure was expressed as a reduction in peak pressure development and coronary flow declined progressively as failure insued. Flow could be maintained at various rates between 40 and 0 % of control by providing minimum levels of dia-

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stolic aortic perfusion pressures after ventricular failure developed.

Hearts were perfused with Krebs-Henseleit bicarbonate buffer containing 11 mM glucose and 1 mM palmitate where indicated. The perfusate was gassed with 95 % O_2 5 % CO_2. The perfusate was allowed to pass through the heart only once and oxygen consumption was calculated from the arterial venous difference in PO_2 and rate of coronary flow. *Rates of glycolysis were determined from* ^3H_2O production from either ^3H 2-glucose or ^3H 5-glucose (2). Fatty acid oxidation was estimated from $^{14}CO_2$ production from U ^{14}C palmitate. Tissue levels of metabolic intermediates were determined by standard enzymatic procedures on hearts quickly frozen and extracted in ice cold 6 % perchloric acid (5). Each heart received a 10 min washout perfusion as a Langendorff preparation, 10 min of perfusion as a control working preparation with a preload of 10 cm H_2O left atrial pressure and an afterload of 60 mmHg hydrostatic aortic pressure (6). Ischemia was then induced and perfusion was continued for the times and with the rates of coronary flow indicated in the figures and tables.

RESULTS AND DISCUSSION

Control hearts with coronary flows around 15 ml/min consumed about 30 μmoles O_2/g dry/min (Fig. 1). These hearts were beating at a rate of 240 per min and developed pressure was about 90 mmHg. When coronary flow was restricted to about 5 ml/min the rate of oxygen consumption decreased to about 20 μmoles/g/min. The percent of oxygen extracted from the perfusate was greater at the lower flow (4) and total oxygen consumption decreased by only 30 % even though flow was reduced by 60 %. At the lower flow heart rate decreased to about 175 beats/min and peak developed pressure was 75-80 mmHg. At coronary flows of less than 5 ml/min, oxygen consumption decreased in proportion to flow (Fig. 1) and peak developed pressure decreased in proportion to oxygen supply (7).

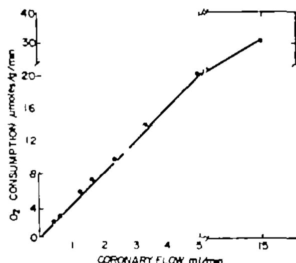


Fig. 1. Effect of reducing coronary flow on oxygen consumption.
Coronary flow was reduced to the levels indicated and oxygen consumption was estimated between the 18 to 20 min of perfusion. The perfusate contained 11 mM glucose, 3 % fraction five bovine serum albumin and 1 mM palmitate.

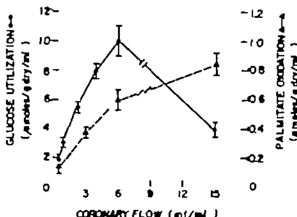


Fig. 2. Effects of coronary flow on glucose utilization and fatty acid oxidation.
The perfusate contained 11 mM glucose and 1 mM palmitate bound to 3 % albumin. Coronary flow was restricted to the levels indicated and rates of substrate utilization were determined over the 15-20 min perfusion period. Glycolysis (solid line) was determined by ^3H_2O production from ^3H -glucose and fatty acid oxidation (dashed line) was determined from $^{14}CO_2$ production from U-^{14}C-palmitate. Each point represent the mean ±S.E.M. for at least 6 hearts.

Glucose utilization was accelerated when coronary flow was restricted by 60 % but this accelerated rate decreased as flow was restricted further (Fig. 2). The fastest rate of glycolysis observed in ischemic hearts, coronary flows around 5 ml/min, was much less than the 15-17 μmoles/g/min that results from hypoxia where coronary flow is maintained at high rates and the arterial PO_2 is reduced (7). These observations emphasize the importance of coronary flow in maintaining not only an adequate supply of oxygen but anaerobic metabolism as well. Fatty acid oxidation decreased in a manner similar to the decrease in oxygen consumption as flow was progressively reduced (Fig. 2). In control hearts the rate of fatty acid oxidation accounted for about 90 % of oxygen consumption. As flow was reduced in hearts receiving 1 mM palmitate, oxidation of

fatty acid still accounted for most of the residual oxidative metabolism that occurred. The presence of fatty acids reduced glucose consumption in control hearts but had little or no effect on glycolysis in the ischemic hearts (3).

Total ATP production was about 180 μmoles/g dry/min in control hearts and 93 % of this was from oxidative metabolism. With a 60 % reduction in flow ATP production was decreased to 170 μmoles/g/min of which 85 % came from oxidative sources. At the lowest rates of flow about 5 % of control ATP production was restricted to about 4 μmoles/g/min with 75 % coming from oxidative sources. Therefore the major source of ATP at all levels of coronary flow remained residual oxidative phosphorylation. Glycolytic production of ATP was about 8.20 and 4 μmoles/g/min at coronary flows of 15.6 and 0.5 ml/min respectively. In comparison, anoxic hearts have maximum rates of glycolytic ATP production from exogenous glucose at about 30-35 μmoles/g/min (7). Therefore if glycolysis could be stimulated in ischemic muscle to the maximum rates seen in anoxic hearts total ATP production could be maintained at normal rates only if oxidative production is not decreased by more than 15 %. This calculation applies only to hearts performing moderate levels of mechanical work such as those used in the present study. Obviously if heart work is increased total ATP utilization will be greater and glycolytic production would compensate for a much smaller percent reduction in oxidative metabolism.

Decreased utilization of glucose resulted from inhibition of glycolysis probably at the level of both phosphofructokinase and glyceraldehyde 3-phosphate dehydrogenase (7,8). At flow rates of 4 ml/min tissue levels of glucose-6-phosphate increased with little change in the levels of fructose 1,6-diphosphate (Fig. 3). Levels of dihydroxyacetone-phosphate also increased. With further reductions in flow glycolysis became progressively more inhibited and levels of glyceraldehyde 3-phosphate, dihydroxyacetone phosphate and fructose 1,6-diphosphate increased in proportion to the restriction in flow. Levels of glucose-6-phosphate remained high at all ischemic rates of flow. These data indicated that glyceraldehyde 3-phosphate dehydrogenase became progressively inhibited as flow was reduced and that the rate of this enzyme restricted overall flux through the glycolytic pathway. This inhibition of glycolysis could not be overcome by treating the tissue with high levels of glucose and insulin (7, 8).

Both lactate and α-glycerol-phosphate accumulated and as calculated from the ratios of α-glycerol-phosphate/dihydroxyacetone phosphate and

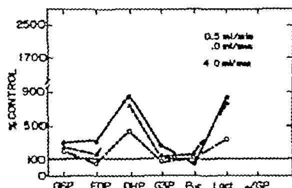


Fig. 3. Effects of coronary flow on tissue levels of glycolytic intermediates.
Hearts were perfused for 20 min at the coronary flows indicated in the figure with perfusate containing 11 mM glucose. Tissue levels of intermediates in ischemic heart are expressed as percent of the levels in control hearts receiving coronary flow of about 15 ml/min. Each point represents the mean of at least 6 determinations.

lactate/pyruvate the NADH/NAD ratio increased. Accumulation of lactate was proportional to the restriction in flow and reached intracellular concentrations as high as 40 mM at the lowest flows (7). Both intracellular and extracellular pH declined to about 6.7 to 6.8 at the lowest coronary flows ( ). Accumulation of lactate and H and perhaps NADH appeared to be major factors responsible from glycolytic inhibition in ischemic tissue (8). When the pH of the arterial perfusate was decreased to 7.0 in anoxic or hypoxic hearts glycolysis was effectively inhibited (8). Buffering the extracellular and intracellular pH in ischemic hearts however results in only minor improvements in glucose utilization. Accumulation of H may contribute to decreased activity of glycolytic enzymes during ischemia but glycolytic inhibition is more complicated than simple changes in tissue pH.

As coronary flow was reduced an inverse relationship was observed between glucose utilization and tissue lactate in hearts perfused with lactate free perfusate (8). Intracellular lactate concentrations of 20-30 mM were associated with maximal glycolytic inhibition. Addition of high levels of lactate (20-40 mM) to the perfusate inhibited glycolysis in aerobic anoxic and in ischemic hearts receiving coronary flows where glycolysis would have otherwise been stimulated. The relationship between tissue lactate and glucose utilization in ischemic hearts receiving 4 ml/min coronary flow is shown in Fig. 4. In hearts perfused with lactate free buffer intracellular lactate was about 10 mM and glucose utilization was about 9 μmoles/g/min. However when lactate was added to the perfusate in concentrations ranging up to 40 mM tissue lactate increased

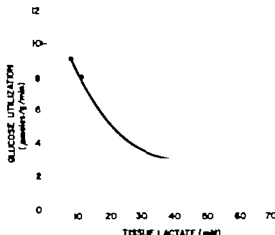


Fig. 4 Effects of lactate on glucose utilization.
Hearts were perfused for 30 min with coronary flows of between 4 and 5 ml/min The perfusate contained 11 mM glucose The tissue lactate concentration was increased by adding Na lactate to the perfusate in concentrations ranging from 0 to 40 mM Intracellular lactate was calculated by substrating extracellular lactate determined from perfusate concentration of lactate and the extracellular space as measured with sorbitol, from total tissue lactate and dividing by the intracellular volume Each point represents the mean of 4-6 determinations

from 10 to as much as 70 mM and glycolysis was inhibited. Maximum inhibition of glycolysis occurred when tissue lactate was 20-30 mM Therefore glycolysis appeared to be maximally inhibited by 20-30 mM lactate whether the lactate was produced from glycolysis and accumulated due to restrictions in coronary flow or accumulated from addition of buffered lactate to the perfusate Addition of Na lactate to the perfusate did not reduce tissue pH Accumulation of lactate independent of changes in cellular pH therefore appeared to be a major factor responsible for inhibition of glycolysis in ischemic heart

Inhibition of fatty acid oxidation appeared to develop at the level of β-oxidation In aerobic control hearts perfused with 1 0 mM palmitate fatty acid oxidation was controlled primarily by the rate of the citric acid cycle which was in turn controlled by the rate of oxidative phosphorylation (9) In ischemic hearts β-oxidation became inhibited as assessed by a large reduction in flux through the pathway (Fig.) and accumulation of high levels of long-chain acyl CoA and acyl carnitine derivatives associated with a large decrease in the levels of both acetyl-CoA and acetyl-carnitine (Table I) Total tissue CoA (free CoASH plus all acyl derivatives) is about 450 nmoles/g dry in rat hearts. In aerobic control hearts acetyl-CoA and long-chain acyl CoA each make up about 30 percent of the total CoA (Table I). In ischemic hearts however long-chain acyl CoA represented about 70 percent of the total and acetyl-CoA decreased to about 3 percent These changes in CoA derivatives can be interpreted as representing inhibition of β-oxidation only if they occurred inside the mitochondrial matrix Approximately 90 percent of total tissue CoA is located in the mitochondrial matrix (10) Therefore the large changes in the acyl CoA derivatives in ischemic hearts probably occurred in the mitochondrial matrix and could be interpreted as representing inhibition of β-oxidation. Inhibition of this process probably developed because of a high mitochondrial NADH/NAD ratio as oxygen supply was restricted β-oxidation becomes inhibited at high levels of NADH in liver mitochondria (11). Flux through the citric acid cycle is also controlled by the NADH/NAD ratio (1 13) and in ischemic hearts flux through the cycle most likely depends on the rate of NADH removal via residual oxidative phosphorylation

High levels of fatty acids may be detrimental to ischemic tissue for several reasons Inhibition of β-oxidation may interfere with ATP production High levels of long-chain acyl CoA comparable to those seen in ischemic tissue inhibits adenine nucleotide translocation across the inner mitochondrial membrane (14) and may interfere with oxidative phosphorylation of ADP When present at high levels oxidation of fatty acids accounts for the majority of oxygen consumption even in ischemic tissue and the pyruvate formed from glycolysis is diverted to lactate When fatty acids are oxidized through β-oxidation formation of each acetyl-CoA results in the production of one FADH and one NADH

Table I Effect of ischemia on tissue levels of acyl CoA and carnitine derivatives.

	Acetyl-CoA	Long-chain acyl CoA	Acetyl Carnitine	Long-chain acyl carnitine
Control	168 9	150 ± 4	1707 ± 166	872 ± 56
Ischemic	22 1	293 11	925 ± 57	3078 ± 143

Hearts were perfused for 20 min with buffer containing 11 mM glucose and 1 0 mM palmitate Coronary flows averaged 13 and 5 ml/min in the control and ischemic hearts, respectively From 6-10 hearts were analyzed for each perfusion condition. All values are expressed in nmoles/g dry weight

These reducing equivalents can be used to support electron transport and would, therefore reduce the dependence on citric acid cycle production of NADH Since the citric acid cycle forms one high energy phosphate at the succinic thiokinase reaction per acetyl-CoA oxidized it would be better to have all of the reducing equivalents formed by the cycle and not by β-oxidation. In addition oxidation of NADH results in three ATP per oxygen atom used whereas oxidation of FADH from β-oxidation produces only two ATP per oxygen used. Therefore oxidation of fatty acids results in less efficient use of oxygen and oxygen consumption is increased by about 15 % in hearts oxidizing fatty acids compared to carbohydrate (9) More efficient production of ATP in ischemic tissue might be accomplished by inhibiting fatty acid uptake or activation and allowing the tissue to oxidize pyruvate from glycolysis In addition to more efficient use of the residual oxygen that is available levels of long chain acyl CoA might be reduced and pyruvate which would otherwise be converted to lactate would be oxidized

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DISCUSSION

Dr Hjalmarson

Thank you Dr Neely for your presentation of these very basic and very important data. We need to understand the metabolic changes induced by ischemia before we can talk about factors that will change the degree of ischemia.

Dr Sobel

What happens to myocardial mechanics in anoxic myocardium in the absence of acute infarction if lactate is added to the perfusion medium Does the presence of lactate *per se* affect decline of ATP in that situation

Dr Neely

In the anoxic preparation, mechanical performance is almost nonexistent and ATP levels are very low Addition of lactate to these hearts does not appear to have any effect on the already depressed state In the ischemic hearts high levels of lactate do appear to decrease mechanical performance but we have not examined this very carefully yet.

Dr Sobel

Did you provide lactate in the perfusion medium from the outset Does the anoxic heart use any of

the lactate or does the extent of anaerobiosis preclude any utilization of lactate

Dr Neely

Yes lactate is utilized, at least in the control hearts These hearts were all perfused with lactate for 10 minutes before starting the anoxic or ischemic perfusion The control heart oxidizes lactate and inhibition of glycolysis under aerobic conditions is very much like the inhibition that develops when fatty acids are oxidized Lactate results in high levels of citrate and glycolytic inhibition is due to decreased activity of phosphofructokinase Tissue levels of ATP are not increased, however

Dr Just

Would you mind commenting on the factors related to irreversibility of glycolytic inhibition and the duration of hypoxia and reduced coronary flow that is required for irreversibility

Dr Neel

It is possible to reverse the glycolytic inhibition if coronary flow is restored after up to about 40 minutes of perfusion at low blood flow However only a small increase in the steady state rate of glycolysis is observed I think this is because oxygen delivery is restored as coronary flow is returned to normal and oxidative metabolism prevents a large increase in glycolysis as happens in control hearts It would be interesting to restore flow with perfusate gassed with nitrogen to see if the anoxic rate of glycolysis can be produced after various periods of ischemia

Dr Braun ald

I think that the observation that ischemia inhibits glycolysis to a greater extent than anoxia does is an extremely important one In trying to relate this to the whole heart either in experimental animals or in patients it is worth recognizing that no matter how hard you try it is very difficult to diminish coronary flow to the same extent as in the isolated perfused heart preparation Studies with microspheres have shown that we cannot achieve such reductions of coronary flow For example the last point that you had Dr Neely showed coronary flow at 4 per cent of control flow which we cannot even approach in the intact organism Thus when we talk about ischemia clinically we are talking about relative ischemia. We do not have as pure a preparation and we are dealing with flows of the order of 10-40 per cent of control and that is a range in which substantial glycolysis can still take place

Dr Neely

Yes at 40 per cent of control flow glycolysis is stimulated but only if there is low arterial lactate In other models where whole blood is used a substantial amount of lactate will be present in the blood and glycolysis probably will not be stimulated This is true in Jim Liedtke s swine heart preparation where arterial lactate ranges from 5 to 10 mM Also other people using microspheres such as Dr Jennings, do report flows as low as 5 per cent of control in certain areas of the ischemic zone

Dr Hjalmarson

When you say you reduce coronary flow to 5 ml per minute in most of your slides are those hearts paced or non-paced

Dr Neely

Most of the data are from non-paced hearts. In some cases we paced the hearts and maintained flow by use of a constant flow pump connected to the aorta.

D Hjalmarson

I remember a couple of years ago you showed a slide where there was an increase in glycolysis when you induced ischemia and this increase was followed by inhibition. Is the initial increase not true any longer or did you not put that peak on your slide?

Dr Neely

That initial increase you referred to was due to rapid utilization of tissue glycogen.The data I presented today were for utilization of exogenous glucose after 20 minutes of ischemia. All the tissue glycogen is used in about 10 minutes after the start of ischemia and breakdown of glycogen would not contribute to glycolysis after this time

Dr Hjalmarson

When long-chain acyl-CoA accumulates you say this will be either in the mitochondria or bound to the outer surfaces of membranes. What happens to fatty acid uptake in severe ischemia?

Dr Neely

Fatty acid uptake still occurs in the ischemic tissue but the rate is decreased. The rate of uptake probably depends on the rate of acyl-CoA removal by oxidative metabolism and the availability of free CoASH. One of the more interesting aspects of β-oxidation inhibition is the accumulation of high levels of long-chain acyl-CoA and the possible effects of this material on other metabolic processes. Dr Shrago's group has demonstrated acyl-CoA inhibition of adenine nucleotide translocase. Perhaps other enzymes are also inhibited.

Dr Hjalmarson

This might suggest that lowering the concentration of fatty acids would result in less acyl-CoA and be beneficial by keeping ATP production going.

Dr Neely

Yes. The only problem is that even if hearts are perfused with fatty acid free perfusate, long-chain acyl-CoA still accumulates. The level is not quite as high as when fatty acids are present, but enough fatty acid is released from tissue lipids to raise long-chain acyl-CoA to about 200 μmoles/g dry compared to 300 when fatty acid is present in the perfusate.

THE ROLE OF MYOCARDIAL MEMBRANE LIPIDS IN THE DEVELOPMENT OF CARDIAC NECROSIS

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SUMMARY

1. The fatty acid composition of cardiac membrane lipids is influenced by age, sex, diet and other factors.

2. The relative amounts of various polyunsaturated fatty acids in cardiac lipids influence markedly the development of myocardial necrosis and mortality following overstimulation with isoproterenol.

3. The availability and metabolism of arachidonic acid may play an important role in regulation of cardiac metabolism.

The purpose of this study is to examine how the chemical composition of cardiac muscle may affect the response of the heart to isoproterenol-stimulation, to energy deficiency and the development of myocardial necrosis.

THE EXPERIMENTAL MODEL

There are important similarities between the two most common models of experimental myocardial necrosis — coronary occlusion and overstimulation with catecholamines. In both instances myocardial necrosis develops as a result of sustained energy imbalance and energy deficit.

Energy deficiency due to impaired energy liberation and oxidation is observed in ischemia, and energy deficiency due to excessive energy utilization and possibly also ischemia is observed following overstimulation with catecholamines, i.e. isoproterenol.

In both instances there is a rapid and large decrease in myocardial levels of high-energy phosphates, ATP and creatine phosphate (CP). There is an important difference in energy depletion of acutely ischemic cardiac muscle following coronary occlusion and in the diminution of energy stores in the non-ischemic but overloaded muscle of the non-infarcted area. Fig. 1 illustrates that when CP is reduced below 7 μmoles/g the ATP is also reduced. The survival limits and lowest levels able to maintain muscle contraction in non-ischemic muscle are 1.5 μmoles/g ATP and 3 μmoles/g CP (1).

In ischemic myocardium there is early inhibition of ATP utilization in the presence of unimpaired utilization of CP stores. Muscle contraction stops at an ATP level of 4.5 μmoles/g when only about 20 % of the ATP has been utilized (1). This suggests an inhibition of ATP transport from mitochondria, but unimpaired utilization of extramitochondrial ATP and energy reserves in form of CP.

The ATP-ADP translocation across the mitochondrial membrane may be a physiological control

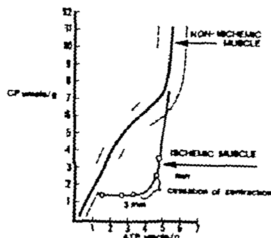


Fig. 1. The relationship between tissue content of creatine-phosphate (CP) and ATP in non-ischemic and ischemic dog heart muscle following coronary artery occlusion. When the creatine phosphate (CP) is reduced below 7 μmoles/g the ATP content is also reduced. The survival limits and lowest levels able to maintain muscle contraction in non-ischemic muscle are 1.5 μmoles/g ATP and 3 μmoles/g CP. Ischemic myocardium. Early inhibition of ATP utilization in the presence of unimpaired utilization of CP stores. Muscle contraction stops at an ATP level of 4.5 μmoles/g.

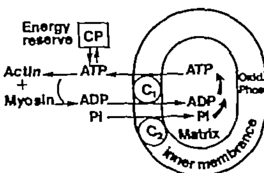


Fig. 2. The ATP-ADP translocation across the inner mitochondrial membrane
Transport of ATP from mitochondria to cytoplasma by carrier C. Stimulation by Ca^{2+} and K^{+}. Inhibition by FA-CoA. Ischemic myocardium. Early inhibition of ATP transport, (possibly due to FA-CoA and intracellular fatty acids).

step in myocardial energy metabolism. This transport of ATP (Fig. 2) is stimulated by Ca^{2+} and K^{+} and inhibited by fatty acids and particularly by certain fatty acyl-CoA derivatives such as oleyl-CoA (2, 3).

In the isoproterenol-model of myocardial necrosis there is a 50 % loss of ATP and 85 % loss of CP 7 hours after injection of isoproterenol 30 mg/kg, according to the studies of Fleckenstein *et al* (4). Partial recovery of the high-energy phosphates occurred 4-6 hours after the injection.

In the isoproterenol-stimulated heart muscle the ATP-CP relationship suggests also such an inhibition of ATP transport but at a later stage 20-30 min after injection of the catecholamine (Fig. 3). In this model of myocardial necrosis there is a significant utilization or diminution of cellular ATP before this apparent inhibition of cellular energy transport takes place.

These two models of myocardial necrosis have both an extensive and longlasting diminution of myocardial high-energy phosphates which leads to progressive cell death and necrosis.

There seem to be many ways to modify the chemical composition of cardiac muscle: nutritional, hormonal, pharmacological and possibly environmental means.

Dietary fat and serum lipids are considered risk factors in cardiovascular diseases and we therefore choose to attempt to modify the lipid composition of cardiac muscle.

The experiments were carried out on male Wistar rats which were divided into several groups.

I Control animals fed *ad libitum* a standard commercial rat chow (KFK V by J. Denmark).

II Animals fed a diet containing 10 % cod liver oil. The animals were kept on this diet for at least 3 months before experimentation.

III Animals fed a diet containing 10 % cod liver oil as group II. This group was injected intramuscularly with α-tocopherol 20 mg once a week.

IV Animals on standard diet and injected intramuscularly with α-tocopherol 20 mg once a week.

The various groups of animals were studied with or without isoproterenol-treatment. Isoproterenol 20-40 mg/kg was injected twice according to the method of Rona (5) the second injection 4 hours after the first injection. The surviving animals were then killed 48 hours after the first injection, the heart was rapidly removed and washed with ice-cold saline.

Myocardial lipids were extracted with chloroform-methanol (2:1) (6). The extraction, separation and saponification of the lipids were carried out in presence of the antioxidant BHT to prevent autooxidation of polyunsaturated fatty acids. The extracted lipids were separated into free fatty acids (FFA), neutral lipids (TG) and phospholipids (PL) by silicic acid column chromatography and then analyzed by gas liquid chromatography (7, 8).

The quality of separation was tested by TLC-chromatography of lipid fractions. The sepa

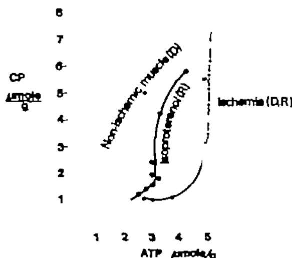


Fig. 3. The relationship between tissue content of CP and ATP in non-ischemic and ischemic dog heart muscle (D) and in ischemic and isoproterenol-stimulated (30 mg/kg) rat heart muscle (R).

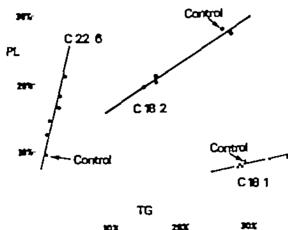


Fig 4 The relationship between fatty acid composition of phospholipids and triglycerides in heart muscle

ration and recovery of each sample was also estimated with the aid of an internal standard i.e. a known amount of heptadecanoic methyl ester or C_{17}-fatty acid.

Calculations of the percentage distribution of methyl esters were performed by electronic integration by Varian integrator and by triangulation of area peaks.

INFLUENCE OF DIETARY LIPIDS ON CARDIAC LIPIDS

Fig. 4 illustrates the relationship between fatty acid composition of phospholipids and triglycerides in heart muscle The fatty acid composition of cardiac lipids, both neutral lipids and phospholipids, is altered in animals on a diet containing cod li er oil. The most significant changes in neutral lipids are an increase in oleic acid and decrease in linoleic acid, in phospholipids there is an increase in docosahexaenoic acid and decrease in linoleic acid.

The availability of polyunsaturated fatty acids influences the composition of phospholipids to a much greater extent and Fig. 4 illustrates the preferred incorporation of more unsaturated fatty acids into phospholipids. Increased availability of docosahexaenoic acid $C_{22:6}$, results i a significant increase in the content of this fatty acid in phospholipids but only in a small increase in the content of C_{22} in glycerides. The linoleic acid is distributed almost equally between phospholipids and glycerides whereas oleic acid seems to be rejected by phospholipids and preferably incorporated into glycerides.

Fig 5 illustrates the inverse relationship between linoleic acid $C_{18:2}$ and docosahexaenoic acid $C_{22:6}$ in phospholipids of rat heart muscle This figure illustrates how the longer chained and more unsaturated docosahexaenoic acid replaces the shorter and more saturated linoleic acid i phospholipids

These observations illustrate how dietary lipids modify both glycerides and phospholipids in heart muscle The unsaturated fatty acids compete for incorporation into phospholipids and the more unsaturated fatty acids seem to be able to displace less unsaturated fatty acids (9)

The next question to be examined is the following: How do cardiac membrane lipids influence the development of myocardial necrosis

INFLUENCE OF CARDIAC LIPIDS ON THE DEVELOPMENT OF MYOCARDIAL NECROSIS

The results in Table I show the fatty acid composition of heart muscle phospholipids before and after development of myocardial necrosis induced by isoproterenol-stimulation 40 mg/kg. Three groups of animals are represented control animals animals fed cod liver oil and animals injected i.m with α-tocopherol 70 units per week for six weeks

The relati e amounts of palmitic stearic and oleic acids are the same in these groups of animals whereas the phospholipid differ with respect to the relative content of polyenoic acids e. $C_{18:2}$ C_{20} and C_{22} Animals fed cod liver oil have considerably less amount of the ω-6 polyenoic acids, e

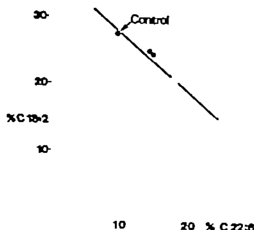


Fig. 5 The relationship between linoleic acid, $C_{18:2}$ and docosahexaenoic acid, C_{22} in phospholipids of heart muscle

Table 1 The fatty acid composition of heart muscle phospholipids before and after development of myocardial necrosis induced by isoproterenol (40 mg/kg)

% FA	Control Before	Control After	Animals fed CLO Before	Animals fed CLO After	Tocopherol Before	Tocopherol After
C 16 0	12.9	13.5	12.1	10.9	12.7	13.1
C 18 0	20.5	22.3	21 9	1.3	21.3	22.3
C 18 1	8.2	9 1	8.5	10.1	7 9	9 4
C 18 2	27 7	30 4	20 1	20 6	20 0	27.8
C 20.4	16 6	13 1	11 1	9.4	20.3	11 6
C 22-6	9.6	9.3	16.4	19 2	13 1	10 7
$\sum \omega$-6	44.5	43.8	32.1	31.3	41.2	39 4
$\sum \omega$ 3	11 0	11.0	19 9	[illegible]	14 4	12.6
ω-6/ω-3	4.05	3 98	1 61	1.24	[illegible]86	3 13

In animals fed cod liver oil (CLO) certain amount of C_{22} may also be present and appear as C_{20} on GLC since these fatty acids are not separated

$C_{18:2}$ and C_{20} and more of the ω-3 fatty acids primarily C_{22} but also C_{20} and $C_{22:5}$. Animals receiving α-tocopherol do show an increase in C_{20} and decrease in $C_{18:2}$

Following isoproterenol-stimulation the surviving animals were killed 48 hours after the first injection of isoproterenol The animals fed cod liver oil usually died before or shortly after the second injection

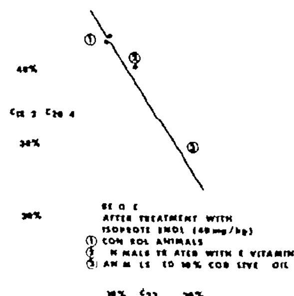


Fig 6 The relationship between the relativ amount of the ω-6 fatty acids (linoleic acid C and arachidonic acid C_{20} and the ω-3 fatty acid docosahexaenoic acid C_{22} in phospholipid of heart muscle

of isoproterenol i e 20-30 hours after the first injection and none of them survived 48 hours after the first injection. These animals had extensive myocardial necrosis Animals receiving vitamin E have on the other hand considerably less necrosis and lower mortality than the other animals

Fig. 6 illustrates a relationship between the ω-6 polyenoic fatty acids i.e linoleic and arachidonic acid and the ω 3 polyenoic docosahexaenoic acid $C_{22:6}$. An inverse relationship exists between the ω-6 and ω-3 f tty acids suggesting a competition between these fatty acid in heart muscle phospholipids.

The fatty acid that seems to be of particular interest with respect to cardiac necrosis and mortality i arachidonic acid (Fig. 7) The relative content of this fatty acid is lower in animals fed cod liver oil and these animals have a 100 % mortality at isoproterenol levels from 20-80 mg/kg. Animal receiving vitamin E have an arachidonic acid level almost twice as high as the relativ e level of $C_{20:4}$ in animals fed cod liver oil Animals receiving vitamin E have much lower mortality than the control group i.e 25 % compared to 57 % in the control group

The change in arachidonic acid level during overstimulation may also be of significance (Fig. 7 lower part) In the group with the lowest mortality the diminution or possibly the utilization of C_{20} is greatest, whereas the animals with the highest mortality show little alteration in C_{20} of heart muscle phospholipids.

Fig. 8 illustrates again a relationship between the relative content of C_{20} in heart muscle phospholipid and mortality of animal following overstimulation with isoproterenol The effect of vitamin E to

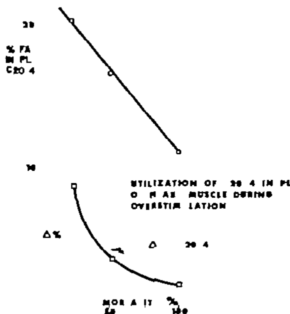


Fig. 7 Upper part: The relationship between the relative content of arachidonic acid, C_{20} in phospholipids of heart muscle and mortality following isoproterenol-stimulation
Lower part Diminution or change in the relative amount (Δ%) of arachidonic acid, C_{20} in phospholipids of heart muscle during stimulation with isoproterenol.

increase the arachidonic acid level and reduce mortality following overstimulation is not merely a function of it property as an antioxidant When animals, fed a diet containing 10 % cod liver oil were injected with vitamin E there was neither an increase in C_{20} levels of phospholipids nor was there a decrease in mortality following stimulation (10)

These observations suggest that there might be a relationship between the composition of membrane phospholipids and the severity of tissue damage in heart muscle following overstimulation with isoproterenol Arachidonic acid appears to play an important role in prevention of cardiac necrosis and the next question is then What is the function of arachidonic acid in cardiac metabolism

METABOLISM OF ARACHIDONIC ACID

Arachidonic acid is synthesized from linoleic acid C_{18} ω-6 by a series of desaturations and elongation. Arachidonic acid is present in tissues, primarily in position of phospholipids. The best known function of arachidonic acid is to serve as a substrate for prostaglandin synthesis (11) Catecholamines are known to stimulate PGE_2 synthesis which in turn serves as regulator of intracellular lipolytic activity and numerous other processes (1 13).

A variety of polyenoic acids, mostly ω-6 fatty acids can serve as substrates for PG-synthesis whereas ω 3 polyenoic acids (22.6 ...5 20:5) do not serve as substrates (14) Unsaturated fatty acids which are not precursors for prostaglandin formation but are present in cellular lipids may be released during lipolysis along with the desired precursor and inhibit PG-formation. The nature and magnitude of this inhibition would then depend upon the composition of acids released. The ω-3 fatty acids which are not substrates can still com-

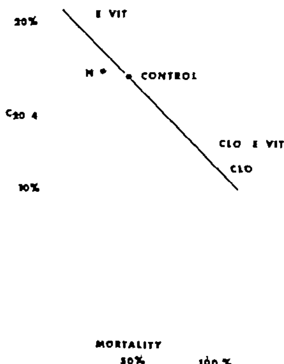


Fig 8. Relationship between the relative content of arachidonic acid, C_{20} in phospholipids of heart muscle and mortality of various groups of animals following stimulation with isoproterenol.
E-vit = animals injected with vitamin E, 20 mg per week for 6 weeks.
N = animals subjected to chronic administration of nicotine 0 5 mg/kg twice day for 6 months.
CLO = animals fed a diet containing 10 % cod liver oil.
CLO + E vit = animals fed diet containing 10 % cod liver oil and treated with the biological antioxidant vitamin E, 20 mg per week

2.0

$\frac{C_{20:4}}{C_{22:6}}$

1.0

0.5

MORTALITY

50% **100%**

Fig. 9 Relationship between the ratio of arachidonic acid, $C_{20:4}$, to docosahexaenoic acid, $C_{22:6}$, in phospholipids of heart muscle and the mortality of various groups of animals subjected to stimulation with isoproterenol 40 mg/kg

pete for binding at the substrate site of the oxygenase and this competitive binding is stronger for more highly unsaturated acids (14)

Our observations suggest that such a competition may be important in these experiments. Fig. 9 shows the mortality increase of stimulated animals with decrease in the ratio of $C_{20:4}/C_{22:6}$. When arachidonic acid is replaced by C_{22} there is a decrease in substrate availability and an increase in inhibitor of PG synthesis. These observations suggest that endogenous prostaglandins may play an important regulatory role in cardiac muscle. Prostaglandins are not the only products formed from 20:4 in heart muscle (Fig. 10). Following the lead of Hamberg and Samuelsson on the transformations of arachidonic acid in human platelets (15) we have examined in a similar manner the transformations of [illegible] labeled arachidonic acid by cardiac microsomes. Several products are formed: PGE, endoperoxides, hydroxypolyene-fatty acids and artifacts of degradation products. The major product (60 %) appears to be hydroxy polyene-fatty acid and PGE_2 is about 15 % of the products.

The functions of these endoperoxides and hydroxypolyene-fatty acids are unknown.

STUDIES ON HUMAN HEART MUSCLE

Preliminary results on human cardiac muscle (autopsy material) indicate that the relative amounts of polyunsaturated fatty acids in myocardial phospholipids may be a function of age and sex.

Heart muscle samples were obtained from accident victims and if these were on microscopic examination without any lesions or abnormalities they were considered normal controls. Samples were also obtained from heart muscle of patients who died of coronary artery disease and from those who died suddenly and were considered sudden cardiac deaths, most often without previous history of coronary artery disease.

Analyses of human autopsy material have to be viewed with caution because of the autolytic changes that take place in the tissue from the time of death until time of sampling, often 20-36 hours. The autolytic changes in heart muscle are relatively slow compared to many other tissues such as liver. The absolute amounts of the various lipid classes change due to autolysis but the relative amounts of the polyunsaturated fatty acids seem to be similar in the various lipid fractions (Table II).

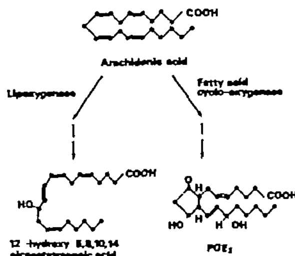


Fig. 10 Transformations of arachidonic acid by rat heart microsomes. Intermediary product such as 12-hydroperoxy 5,8,10,14-eicosatetraenoic acid or prostaglandin endoperoxide are not indicated

Table _ The relative amount of C_{20} and ω 3 fatty acid in lipid fractions of human heart muscle

		FFA			PL			TG		
		% 20 4	% Σω-3	20 4 / ω-3	% 20 4	% Σω-3	20 4 / ω-3	% 20 4	% Σω-3	20 4 / ω-3
Man 63 y	)	_5.5	16.0	1 6	15 1	8 9	1 7	8.1	5.2	1.6
	b)	4 1	17 6	1 4	17 1	8 5	_0	6 7	5 4	1_
Woman 69 y	)	4 3	6 5	3 7	20.0	6 3	3_	6.8	2.4	2.8
	b)	4 3	6 9	3 5	18_	6.1	3 0	9 8	3 1	3.2

20 4 = Arachidonic acid
Σω-3 = Σ20.5 + 22 5 + 22 6

In the FFA fraction the C_{20} makes up about one fourth (1/4) of the total FFA in phosphol pids the C_{20} is 15-17 % of total fatty acid and in neutral lipids it is 6-8 % of total The ratio 20·4/ω 3 is however simila in all three lipid fractions The same ratio of 20 4/ω 3 in FFA and esterified fatty acid fractions suggest that these free fatty acids deri e f om lipolysis of PL and TG The elatively large portion of FFA consisting of 20 4 and ω 3 fatty acid suggests a preferential elease of the polyunsaturated fatty acids and that this elease is non-selective with respect to 20·4 and ω 3 Multiple sampling from different areas of the heart suggests also regional differences in cardiac l pid composition

Fig. 11 illustrates the relationship between the ω-6, the essential fatty acids and the ω-3 the non-essential fatty acids in phospholipids of human heart muscle We observe here as in the rat heart an inverse lationship between these two types of polyunsaturated fatty acids In older individuals the ω 3 fatty acids appeared to be replacing more of the ω-6 fatty acids in the phospholipids The relative amounts of these fatty acids i.e the ratio ω-6/ω 3 change as a function of age (Fig. 12) In a young man of 17 this ratio of ω-6/ω-3 is about 6 1 and diminishes until age 50 and remains relatively constant after that, at a ratio of 3 1

In the female heart this ratio appears to be higher and to diminish with age in the same manner ob-

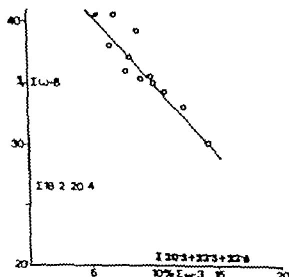


Fig 11 The relationship bet een ω-6 polyunsaturated fatty acids and the ω-3 non-essential polyunsaturated fatty acids in phospholipid of human heart muscle

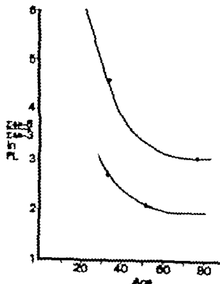


Fig. 12. The relative amounts of ω-6 to ω-3 polyunsaturated fatty acids in phospholipids of human heart muscle in relation to age.

served in heart muscle of men (Fig. 13). In the heart muscle of men who died sudden cardiac death the ratio ω-6/ω-3 is considerably higher in most instances than would be expected for their age group (Fig. 14).

When we examine the relative amounts of the prostaglandin substrate arachidonic acid the prostaglandin inhibitors and the ω-3 fatty acids we observe that the ratio 20:4/ω-3 is also a function of age (Fig. 15). The ratio decreases from age 17 to the age of 50 and remains relatively constant after that.

In those men who died suddenly this ratio is exceptionally high in 5 of 8 cases (Fig. 16). The remaining three cases follow the relationship expected for their age. The heart muscle of patients who died from coronary atherosclerosis showed the relative amounts of 20:4/ω-3 or ω-6/ω-3 expected for their age.

These data suggest that the polyunsaturated fatty acid of the ω-6 and the ω-3 families may be of importance for myocardial metabolism and the preservation of cardiac muscle. In the rat heart the availability of 20:4 seems to be of importance in the isoproterenol-stimulated heart. A replacement of 20:4 by the ω-3 fatty acids increases the development of myocardial necrosis and mortality following stimulation. In human heart muscle sudden

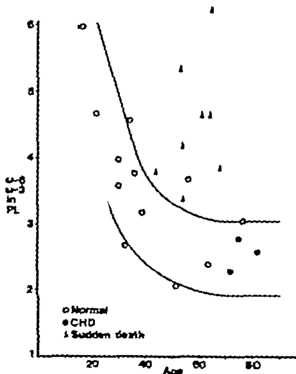


Fig. 14. In the heart muscle of men who died sudden cardiac death the ratio ω-6/ω-3 is higher in most instances than would be expected for their age group.

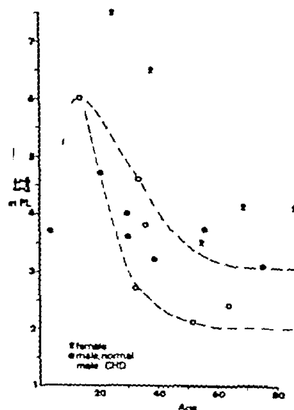


Fig. 13. The relative amount of ω-6 to ω-3 polyunsaturated fatty acids in phospholipid of human heart muscle in relation to age. In the female heart the ratio ω-6/ω-3 is higher than in the male heart. In the female heart this ratio diminishes in the same manner observed in heart muscle of men.

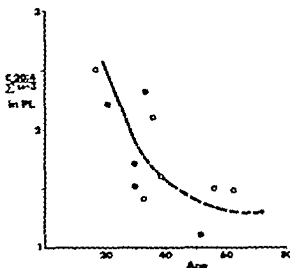


Fig. 15. The relative amount of arachidonic acid C_{20} to ω-3 fatty acids in relation to age (human heart muscle).

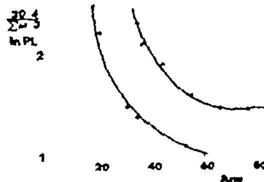


Fig 16 In the heart muscle of men who died sudden cardiac death the ratio $C_{20:4}/\omega$-3 is very high in 3 of 6 cases The remaining three cases follow the relationship expected for their age The heart muscle of patients who died from coronary atherosclerosis (CHD) showed the relative amounts of $C_{20:4}/\omega$-3 expected for their age

death is often accompanied by a very high ratio of 20:4/ω-3 This suggests a different kind of abnormality in the human heart possibly a diminution in the utilization of 20:4 the arachidonic acid

The consequences of diminished availability or diminished utilization of arachidonic acid would be

I Impaired prostaglandin synthesis and
   a) defective control of cellular lipolytic activity
   b) defective control of norepinephrine release from nerve terminals in cardiac muscle

II Impaired synthesis of other products such as endoperoxides and hydroxypolyene-fatty acids, which may have a role to play in regulation of myocardial metabolism

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DISCUSSION

Dr Hjalmarson

Thank you very much I think this is very interest ing and supports some old observations about how dangerous some fatty acids can be for the heart I think of erucic acid and we have to know much more about that What kind of evidence do you have that it is a change in myocardial metabolism that will make these animals more sensitive to isoproterenol Might it not be that you change pla telet functions and get more aggregation when you give isoproterenol? I mean could it be an extra-cardiac effect

Dr Gudbjarnason

There are certainly many numbers of possibilities What is the cause and what is the effect is often difficult to differentiate From the physiological or the histological examinations in those sections there did not seem to be any malfunction in the vascular system This study was primarily directed at the question of the involvement of the muscle it self We do not have any evidence for vascular in olvement, but we cannot exclude them

Dr Hjalmarson

I wonder if you have checked the cardiac metabolism in any other way than just sensitivity to high dosage of isoproterenol Have you measured func tion in some way

Dr G dhjarna on

No we did not We made a great deal of effort to f nd way to quantitate the injury We attempted to use enzymatic methods the incorporation of labeled sulfate and a number of things. Many of those methods that have been employed successfully with other models such a the model of coronary occlusion did not seem to work well in this particular model For example the CPK changes seem to show up much later in this model than in the model of coronary occlusion There are certain difficulties in comparing these model directly although there are important similarities. We did not measure energy metabolism and we did not measure function

Dr Hjalmarson

Since so much in the heart is regulated over membranes the changes of the membranes are very important. There is a need to get some studies where you simply perfuse a heart as Dr Neely showed where you have changes in the membrane content of phospholipids.

Dr Pedersen

The overmortality you demonstrated in connection with different variations in the cardiac metabolism – could you specify how they were distributed on different causes of death I mean cardiac arrest, cardiogenic shock or cardiac deficiency Is that possible in your animal experiments? Especially how much of the overmortality in after-stimulation with isoproterenol was caused by ventricular fibrillation directly precipitated by isoproterenol?

Dr Gudbjarnason

We observed arrhythmias frequently in animals in the high mortality groups but these observations were only continued for a limited period of time usually a few hours after injection of isoproterenol The animals died later and we do not know how they died if by cardiac arrest or fibrillation

Dr Powpa

What do you think about the role of prostaglandin in the creation of necrosis? It was suggested that the release of prostaglandin accelerates the aggregation of platelets Now if you have more free prostaglandin the chance to aggregate platelets increases What is your opinion about it?

Dr Gudbjarnason

It is important to distinguish between endogenous and exogenous prostaglandins The endogenous prostaglandins are synthesized at the membrane surface in cell by microsomal enzymes. We have no evidence for an involvement of myocardial prostaglandins in platelet aggregation

Dr Powpa

But what you showed here concerns the endoge nou prostaglandin And this seems to be released by O--lack

Dr Gudbjarnason

We did not make any direct measurements of prostaglandins. We measure the relative amounts of fatty acids in cardiac phospholipids and the data suggest a possible involvement of prostaglandins in the reaction of heart muscle to isoproterenol-stimulation. The depletion of arachidonic acid from phospholipids of the stimulated heart is of interest and the small decrease in arachidonic acid in phospholipids of animals with high mortality. The high ratio of arachidonic acid to omega 3 fatty acids in heart muscle of those who die a sudden cardiac death suggests a direction where we might look for further information.

EFFECT OF REDUCTION OF MYOCARDIAL FREE FATTY ACID METABOLISM RELATIVE TO THAT OF GLUCOSE ON THE ISCHEMIC INJURY DURING EXPERIMENTAL CORONARY ARTERY OCCLUSION IN DOGS

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Institute of Medical Biology
University of Tromsø Norway

Acute myocardial infarction (AMI) in man is accompanied by an early rise in the plasma concentration of free fatty acids (FFA) probably due to increased adipose tissue lipolysis as a result of enhanced sympatho-adrenal activity (5 10 34). Those patients with the highest plasma FFA concentrations have been reported to be at the greatest risk of developing serious ventricular arrhythmias and death (7 22) although not all investigations have confirmed this association (21 25). Similar increases in plasma FFA concentration have been reported to increase the frequency of ventricular arrhythmias (11) and the severity of myocardial ischemic injury (9) during experimental coronary occlusion in dogs. In healthy dogs elevated plasma FFA enhances myocardial oxygen consumption ($M\dot{V}O_2$) without improving the mechanical activity of the heart (17 18) suggesting that the deleterious effect of FFA during coronary occlusion may reflect an increase in the oxygen requirement of the ischemic tissue

Such observations have raised the possibility that the severity of acute myocardial ischemic injury may be limited by measures which decrease the delivery of FFA to the ischemic cells. This can be done by 1) antilipolytic agents i.e. agents which inhibit catecholamine-induced lipolysis in adipose tissue with a fall in plasma concentrations and myocardial uptake of FFA 2) by infusion of albumin to increase the binding capacity of FFA in plasma thus reducing myocardial uptake of FFA 3) by agents that favour glucose metabolism of the heart relative to that of FFA e.g. sodium dichloroacetate or GIK (glucose-insulin-potassium which will be covered by other contributors at the symposium)

We studied the effect of antilipolytic agents, lipid-free albumin or sodium dichloroacetate on the severity of acute myocardial ischemic injury during experimental coronary artery occlusion in open-chest dogs. The severity of the ischemic injury was assessed quantitatively as the sum of ST segment elevation in epicardial electrocardiographic recordings at 10-15 sites (ΣST). ΣST at 15 minutes occlusion was ordinarily used as an index of the ischemic injury. Previous studies have shown that this technique provides rapid and reproducible determinations of ischemic injury in the same animal (15)

ANTILIPOLYTIC AGENTS

β Pyridyl-carbinol

Firstly the possibility that inhibition of catecholamine-induced lipolysis by a nicotinic acid derivative β-pyridyl-carbinol (Ronicol F Hoffman-La Roche & Co A.G Basel Switzerland) would diminish the magnitude and extent of the myocardial injury after experimental coronary occlusion was studied (9). ΣST during control occlusion alone averaged 41 ± 7 mV (mean ± SEM). Inhibition of lipolytic activity by β-pyridyl-carbinol before repeated coronary occlusion reduced the occlusion-induced ΣST to 21 ± 6 mV ($p < 0.001$). In other experiments isoproterenol intravenously (0... 0.3 μg/kg/min) increased the severity of the ischemic injury. The effect of isoproterenol on occlusion-induced ΣST was markedly reduced however when the lipolytic effect of the drug was abolished by β-pyridyl-carbinol. Furthermore during 4 hours treatment with β-pyridyl-carbinol the extent of depression of myocardial creatine phosphokinase (CPK) activity from full wall biopsies was significantly less than anticipated from the magnitude of ST segment elevation produced by coronary artery occlusion alone. Tissue damage or the infarcted area, was therefore less after inhibition of lipolysis compared to that which would have been expected from the ΣST after the control occlusion.

p-Chlorophenoxyisobutyrate (CPIB)

CPIB or ethyl-CPIB clofibrate (Imperial Chemical Industries Ltd Cheshire U K), has been reported

to reduce plasma FFA concentrations both in animals and in man (1 4 14) although it is not known whether this is accompanied by a reduction in the utilization of FFA by the myocardium (33) as has been demonstrated with β-pyridyl-carbinol and its active metabolite nicotinic acid (12, 18). We have studied the effect of CPIB on both the extraction of FFA by the heart and the severity of acute myocardial ischemic injury during experimental coronary occlusion in dogs (19) ΣST during coronary occlusion alone averaged 26 ± 6 mV Intravenous (i) administration of CPIB 30 minutes before re-occlusion reduced ΣST to 14 ± 3 mV ($p < 0.03$) A continuous i v infusion of isoproterenol increased ΣST to 74 ± 11 mV Pretreatment with CPIB reduced ΣST during isoproterenol infusion to 40 ± 7 mV ($p < 0.005$). CPIB had no effect on mean aortic blood pressure heart rate or regional myocardial blood flow as measured by radioactive microspheres Arterial free fatty acid (FFA) concentrations were reduced by CPIB from 466 ± 41 to —1 ± 44 μEq/l ($p < 0.001$) in the basal state and from 1966 ± 183 to 1479 ± 209 μEq/l ($p < 0.001$) during isoproterenol infusion. The reduction in arterial FFA concentration was associated with a proportionate decrease in the myocardial extraction of FFA Similar changes were observed when CPIB was administered during a pre-existing occlusion which had been established 10 minutes earlier These observations confirm that the severity of acute myocardial ischemic injury in dogs is positively correlated with the myocardial extraction of FFA and can be reduced by effective antilipolytic therapy

Prostaglandin-E (PGE)

Another series of experiments were carried out to study whether a naturally occurring antilipolytic agent PGE₁ would also reduce the severity of the acute ischemic injury in dogs (4) The ability of PGE_1 to antagonize catecholamine-induced adipose tissue lipolysis (31 3) is more marked and consistent than its inhibitory effect on basal lipolysis A complicating factor is that PGE is also a potent vasoactive agent causing peripheral vasodilation with a consequent fall in arterial blood pressure (3) which might increase the size of the myocardial ischemic injury (15) Using a dose of PGE_1 with minimum vasoactive effects, yet with a significant antilipolytic effect on isoproterenol-induced lipolysis studies were done of the effect of PGE on acute myocardial ischemic injury

In nine experiments ΣST was measured during a control occlusion during an occlusion induced 5 min after the start of isoproterenol infusion (0 μg/kg/min i v) and during an occlusion during which the isoproterenol infusion was preceded by a PGE_1 infusion (0 6 μg/kg/min i v) Isoproterenol increased ΣST from 15 4 ± 5 7 mV (mean ± SEM) to 46 ± 8 0 mV ($p < 0.001$). Plasma FFA also increased during isoproterenol from 530 ± 30 μEq/l to 2020 ± 180 μEq/l ($p < 0.001$) Isoproterenol increased heart rate (HR) from 144 ± 7 to 171 ± 5 beats/min ($p < 0.001$) and $\overline{AP}$ decreased from 110 ± 5 to 96 ± 8 mm Hg ($p < 0.01$) The effects of isoproterenol on HR $\overline{AP}$ and on both epicardial and endocardial myocardial blood flow (MBF) of ischemic tissues were unchanged. However PGE reduced ΣST from 46 4 ± 8 0 to 35 6 ± 7 mV ($p < 0.001$) and FFA from 2020 ± 180 to 1390 ± 270 μEq/l ($p < 0.005$)

In another series of eight experiments the effect of PGE_1 was studied by an intravenous infusion 0 6 μg/kg/min or by infusion into the left atrium 0 03 μg/kg/min and i a. did not change ΣST (control 17 3 ± 3 , PGE i v 14 3 ± 4 0 PGE_1 i a. 15 1 ± 3.3 mV) $\overline{AP}$ fell both during PGE_1 i v and i a by 20 % ($p < 0.001$) but HR remained unchanged During these infusions plasma FFA concentration remained unchanged There was a reduction in MBF both in the non-ischemic myocardium and the ischemic myocardium

These results show that PGE reduced the severity of myocardial ischemic injury during isoproterenol infusion and this was associated with a decrease in arterial plasma FFA and therefore presumably with myocardial FFA uptake At basal lipolysis a potential beneficial effect of PGE_1 was outbalanced by the fall in blood pressure so that no change in the size of the ischemic injury was observed.

PGE_1 administration scarcely represents a therapeutic alternative in patients with myocardial infarction in order to reduce the infarct size Its vasoactive properties might in a given situation be so marked as to cause hypotension and shock. The present study is probably more interesting from a pathophysiological point of view since prostaglandins have been proposed to play a role as feed back inhibitors of hormones that act via stimulation of adenyl cyclase (8) Thus the present study might indicate that the balance between prostaglandin and catecholamine activity might be of importance in determining the size of an experimental myocardial infarction

There are several possibilities for the mechanism of the deleterious effect of FFA in the ischemic myocardium Acute coronary occlusion has been shown to stimulate the release of catecholamine stores within the myocardium (26) and this would be expected to enhance the hydrolysis of intramyocardial triglyceride with a local release of FFA (6).

Studies with nicotinic acid (6) and with CPIB (de Deckere E.A.M. O. D. Mjøs and N. E. Miller: Unpublished observations) indicated that these agents inhibit catecholamine-induced lipolysis in the isolated rat heart. Thus, the limitation of myocardial ischemic injury achieved with antilipolytic agents may have been related to an inhibition of intramyocardial lipolysis within the ischemic zone with a local reduction in myocardial oxygen demand as well as to the fall in the arterial concentration of FFA.

In addition to their calorigenic activity FFA have other metabolic actions which might also be related to their deleterious effect during experimental ischemia. Thus the inhibition of glycolysis by FFA in the normally perfused heart (23) raises the possibility that they may also impair carbohydrate utilization during coronary occlusion. More recently it has been proposed that FFA may augment myocardial ischemic injury by increasing the intracellular concentration of long-chain acyl CoA esters which have been shown *in vitro* to inhibit the translocation of adenine nucleotides across the mitochondrial membrane (27) and which are known to accumulate within the heart during experimental coronary occlusion (28).

The results of the present study cannot be interpreted as indicating that β-pyridyl-carbinol or CPIB might similarly reduce the severity of acute myocardial ischemic injury in man. However they strengthen considerably the proposal that effective antilipolytic therapy during the early phase of AMI in man when FFA concentrations may be increased by 3-fold or more (10 22, 34), might be of value in limiting the size of the infarct.

LIPID-FREE ALBUMIN INFUSION

FFA in plasma is bound to albumin and in principle it is the FFA/albumin molar ratio which determines the uptake of FFA in tissues e.g. in the myocardium. In situations with high plasma concentrations and myocardial uptake of FFA the possibility is therefore that an acute increase in the albumin concentration of plasma would reduce FFA/albumin molar ratio in plasma, and consequently reduce myocardial extraction of FFA. If so one might further expect that infusion of albumin would reduce the severity of acute myocardial ischemic injury. This hypothesis was tested in open-chest dogs (Miller N. E. O. D. Mjøs and M. F. Oliver: In preparation).

Table I shows one experiment. High plasma concentrations of FFA were produced by a continuous i.v. infusion of isoproterenol (0.25 µg/kg/min). Fifteen minutes after start of the isoproterenol infusion a branch of the left descending coronary artery was occluded, and plasma concentrations of albumin and FFA myocardial extraction of FFA and ΣST were measured. Then a rapid injection of lipid-free albumin was given intravenously so as to nearly double the plasma albumin concentration, and the same parameters were measured at 2 4 and 6 minutes after the albumin injection. This resulted in a transitory fall in FFA/albumin ratio with a consequent reduction

Table I. Effects of intravenous infusion of lipid-free albumin (0.75 g/kg) during coronary occlusion in a typical experiment, open-chest dog. Mean aortic blood pressure and heart rate were unchanged.

O C C L U S I O N

I S O P R O T E R E N O L

	15	10	Control	+2	+4	+6
ALBUMIN (g/100 ml)			2.1	3.4	3.4	3.4
FFA (µEq/l)			2700	3320	3460	3640
FFA/albumin molar ratio			8.9	6.7	7.0	7.4
FFA extraction (µEq/l)			420	20	+80	+300
ΣST (mV)			177	133	138	146

TIME IN MINUTES

in myocardial extraction of FFA and ΣST or the severity of the ischemic injury was reduced These changes were most pronounced minutes after the lipid free albumin infusion and gradually diminished although being significant both at 4 and 6 minutes

This is the first demonstration in intact animal where an acute decrease in FFA/albumin molar ratio effected a reduction in myocardial extraction of FFA Furthermore the study strengthens the hypothesi that excess FFA i harmful for the ischemic myocard and that interventions which reduce myocardial uptake of FFA might be good for the ischemic heart However a indicated in Table I and demonstrated in other experiments (Miller N. E. O D Mjos and M F Oliver In preparation) the effect of lipid-free albumin lasted for less than 10-15 minutes At that time FFA/albumin molar ratio and myocardial FFA extraction had normalized Therefore acute infusions of lipid free albumin is hardly a therapeutical alternati e in the treatment of acute myocardial infarction in man

SODIUM DICHLOROACETATE

In 196. Lorini and Ciman (13) reported that di-isopropyl-ammonium dichloroacetate raised the respiratory quotient in alloxan-diabetic rats suggesting an incre sed peripheral utilization of glucose relative to that of FFA Stacpoole and Felt (29 30) demonstrated that dichloroacetate (DCA) was the active component and that thi stimulated glucose oxidation and inhibited FFA oxidation in diaphragm muscle from alloxan-diabetic rats. McAllister *et al* (16) confirmed these observations and reported that DCA had simila effects on the metaboli m of the isolated perfused rat heart In the same tudy DCA also antagonized the inhibition of myocardial glucose util zation induced by alloxan-diabetes or by elevation of pla ma FFA concentrations in dogs A sociated changes in lactate and pyruvate metabolism nd in intracellular intermediates suggested that DCA w timulating both the aerobic and anaerobic ut liz tion of glucose by increasing the ac ti ities of phosphofructokinase and pyruvate dehydrogenase An acti ation of pyruvate dehydrogenase by DCA in rat myocardium was subsequently confirmed by Whitehouse and Randle (35)

In the present tudy we ha e tigated further the metabol c nd haemodynami effects of DCA in dogs and ha e e amined the possibility th t the timulation of myocardial gluc se ut liz tion by DCA might limit the se erity of ute my c rdial ischemic injury d ring experiment l oronary occlusion (20)

The results showed that DCA effectively reduced the degree of ST segment elevation induced by subsequent coronary occlusion both under basal conditions and during a continuous intravenous infusion of isoproterenol A similar result was obtained when DCA was given during an established coronary occlusion This effect could not be explained by changes in $\overline{AP}$ HR or myocardial blood flow as measured by radioactive microspheres Measurements in arterial and coronary sinus blood of glucose and FFA concentrations and of FFA radioactivity during ^{3}H palmitate infusions demonstrated an increase in the myocardial utilization of glucose and a decrease in that of FFA Thus this study provides considerable support for the proposal that the survival of the ischemic myocardium may be assisted by measures which enhance the utilization of glucose relative to that of FFA

CONCLUSION

Acute experimental myocardial ischemic injury in dogs can be effectively reduced by agents that reduce myocardial extraction of FFA (antilipolytica lipid free albumin) thus indirectly favouring myocardial glucose metabolism or by agents like sodium dichloroacetate and GIK which seem more di rectly to enhance the utilization of glucose relati e to that of FFA At the present stage in the early treatment of myocardial infarction in man promising studies are under way both in Oslo and in Edinburgh using nicotinic acid analogues

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DISCUSSION

Dr Hjalmarson

Since the papers of Drs Mjos and Kjekshus are closely related I suggest that the two papers will be discussed together

EFFECTS OF LIPOLYTIC AND INOTROPIC STIMULATION ON MYOCARDIAL ISCHEMIC INJURY

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Following acute coronary artery occlusion the level of circulating catecholamines increases many fold reaching the highest levels in patients with extensive infarction and attendant pump failure (1). Exogenous administrations of catecholamines have also been used to support the failing heart and to improve the hemodynamic condition. However the pathophysiological role of catecholamines during myocardial infarction has been controversial.

CATECHOLAMINES AND INFARCT SIZE

Previous studies have demonstrated that catecholamines increase the oxygen requirement of the heart and aggravate symptoms of myocardial ischemia.

The extent and magnitude of an experimental infarction is increased (2) despite concomitant increase in cardiac index. This can be illustrated by studying the opposite and adverse effects exerted by catecholamines on normal and ischemic myocardium (Fig. 1). Occlusion of a coronary artery is followed by increase in diastolic length and bulging in systole in the ischemic area, while myocardial dimensions are unchanged in the oxygenated area. Administration of isoproterenol increases left ventricular contractility indicated by a more rapid rise of intraventricular pressure (dP/dt) with a shortening of myocardial length in non-ischemic areas. In the ischemic area there is a similar but very short lasting reduction in length followed by an increase beyond that obtained before isoproterenol infusion was given, demonstrating the adverse effect obtained in the ischemic myocardium. During graded global myocardial *ischemia isoproterenol may increase cardiac output* until coronary flow has been reduced to 38 % of control values. At this stage of ischemia admin

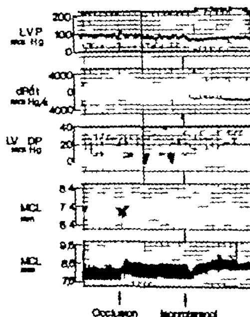




Fig. 1. Effect of isoproterenol on the ischemic myocardium. A branch of the left anterior artery is occluded by a clip. Ultrasound distance gauges measure instantaneous myocardial lengths in the left ventricle.

LVP: Left ventricular pressure
LVDP: End diastolic pressure
dP/dt: The rate of left ventricular pressure rise
MCL: Distance gauges each measuring a chord length of the left ventricle of about 7 to 8 mm, the lower being in the ischemic area, the upper is located outside this area.
Isoproterenol 0.25 µg/kg/min.

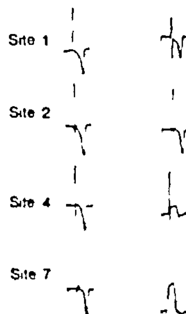
i tration of isoproterenol no longer improves the cardiac output but a precipitous drop in performance is observed (3) Thus it is clear that catecholamines increase the extent of myocardial infarction with further depression of function in the same area albeit the hemodynamic consequences are temporarily offset by the stimulation of the non-ischemic myocardium

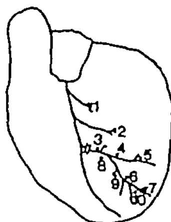
Agents reducing myocardial oxygen consumption i e β-receptor blockade reduce symptoms of myocardial ischemia and have been shown to reduce the size of the ischemic injury in dogs ( ) However β-receptor interventions are associated with depres ion of overall myocardial performance and might therefore precipitate overt cardiac decompensation although functional recovery might take place in the ischemic myocardium (4)

The question therefore arises is it pos ible to improve the oxygen balance in the ischemic myocardium *without interfering with the mechanical* activity of the non-ischemic heart It is now accepted that any increase in myocardial oxygen demand in exces of the coronary oxygen supply will aggravate the myocardial ischemia

FREE FATTY ACIDS AND MYOCARDIAL OXYGEN CONSUMPTION

The increase in myocardial oxygen consumption induced by catecholamines has generally been t tributed to augmentation of the mechanical work of the heart (5) More recent studies have shown that high levels of free fatty acids increase myocardial oxygen consumption without changes in myocardial mechanical activity and that a significant fraction of the increased myocardial oxygen consumption induced by catecholamines is due to their potent lipolytic effect and excessive release of free fatty acids from local and peripheral triglyceride stores (6 7 15) The contribution of free fatty acids to the increase in oxygen consumption induced by catecholamines can be demonstrated by inhibiting the lipolytic activity by nicotinic acid or one of its derivatives This will reduce myocardial oxygen consumption in the catecholamine stimulated heart by as much a 30 % indicating a saving of myocardial energy expenditure (7) The effect on myocardial mechanical activity is not changed by inhibition of lipolytic activity All extra oxygen consumed due to uptake of free fatty acids are dissipated a heat (8) Increased cycling of free fatty acids into tri glycerides may account for some but not all of the increase in energy requirement. More impor tant is probably the role played by free fatty acid as uncoupler of the oxydative phosphorylation which might contribute significantly to the observed rise in oxygen consumption (9) Recent studies have suggested that high levels of free fatty acids inhibit mitochondrial adenine nucleotide translocase activity (10). This might result in lowering of the rate of energy delivery for muscle contraction and electrical conduction in the





F g ... M pping of epicardial ST-segment changes Position 1 and 2 represent sites outside the ischemic area position 4 and 7 represent the ischemic area Epicardial electrocardiograms are show before and during cor onary artery occlusion Not ST-segment elevation

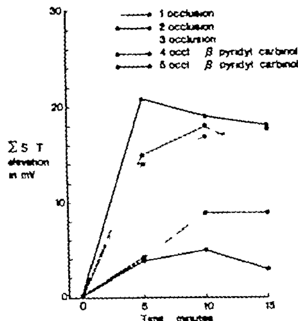




Fig. 3 Effects of coronary occlusion alone and occlusion during infusion of β-pyridyl carbinol (0.1 mg/kg/min). Right panel. lined area, area of ischemic injury after 15 min occlusion stipled area, area showing ST-segment elevation under the influence of β-pyridyl carbinol, LAD left anterior descending coronary artery LA, left atrial appendage LC left circumflex artery Left panel ST – in the same experiment after three simple occlusions and after two occlusions under the influence of β-pyridyl carbinol.

heart and thus indirectly increase the energy requirement.

FREE FATTY ACIDS AND INFARCT SIZE

Interventions designed to change infarct size can be studied in animal models using epicardial multispot mapping of ST-segment changes during short occlusion (15 min) of a coronary artery (2) (Fig. 2) Epicardial ST-segment elevation is a sensitive index of reversible local ischemic injury Provided sustained coronary occlusion it conforms closely with later development of myocardial depletion of CPK in corresponding sites (?), denouncing irreversible cell damage

Repeated coronary occlusions of 15 minutes duration demonstrated clear reduction in epicardial *ST-segment elevation* when *β*-pyridyl carbinol was given to inhibit lipolytic activity (Fig 3). Antilipolytic treatment for 24 hours following coronary occlusion was associated with less CPK depletion in the ischemic area as compared with heart receiving no treatment (12) (Fig. 4) indicating salvage of the ischemic myocardium.

A depressive effect of FFA on isolated hearts has been demonstrated by Henderson Craig *et al* (13) They attributed this to the possibility that the hearts wer oxygen-limited because high concentrations of FFA reduced developed tension in hypoxic rat papillary muscles, but not in the well oxygenated muscle (14) Studies were therefore undertaken by us to test this hypothesis (15) The left coronary artery in dog hearts was perfused through a shunt f om a carotid artery and left ventricular oxygen supply could thereby be reduced by gradual clamping of the shunt line while recording changes n ventricular volume and contractility Elevation of plasma FFA by exogenous administration of a lipid solution and heparin resulted in increased myocardial oxygen consumption in the non-ischemic heart without ventricular dilatation or depression of contractility After myocardial function was depressed by reducing the coronary flow in such a way that a constant but estricted oxygen supply was secured similar administration of FFA to the heart led to further reduction in myocardial mechanical activity and increased lactate production probably due to augmented oxygen requi ement induced by increased FFA uptake and consumption in competition with oxygen needed for mechanical activity

Isoproterenol infusion performed during coronary artery occlusion in order to mimic the increased sympatho-adrenal activity observed in patients with acute coronary occlusion markedly increased plasma concentrations of free fatty acid Concomitantly epicardial ST-segment elevation was clearly augmented and the number of epicardial sites with ischemic ST-changes increased when compared with control occlusion alone Administration of *β*-pyridyl carbinol before isoproterenol was given abolished the lipolytic activity completely (Fig. 5) and markedly blunted the ST-segment elevation following coronary artery occlusion (1).

The effect of isoproterenol on heart rate ventric

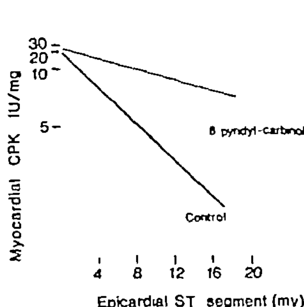


Fig 4 Depression of myocardial CPK activity after administration of β-pyridyl carbinol in animal with coronary artery occlusion. Epicardial recordings were obtained 15 min after coronary artery oc lusion f om anatomically identifiable sites. The artery was then released, and permanently ligated CPK act ity expressed on logarithmic scale w me sured in homogenates f om f ll wall specimens obtained from the same sites 4 hours later. Data from multiple samples f om three animal ecei ng β pyridyl carbinol bef w ligat on and maintained f r 4 hours (open ircles) and f om five ntreated animal (control) (losed circles). Solid line regression line f r control tudy (log CPK 1.300 – 0.067 ST = 0.89). Dotted Line regression line relating ST-segment elevation fter oronary artery oc lusion before admini tration of β pyrid l carbinol nd log CPK from corresponding sites 4 hours later (log CPK = 1.278 – 0.033 ST = 0.78). Animal recei ing β pyridyl carbinol showed les depression of my ocardial CPK acti ity than would ha been pected from ST-segment les now occurring bef e drug administr on (p <0.001) (1)

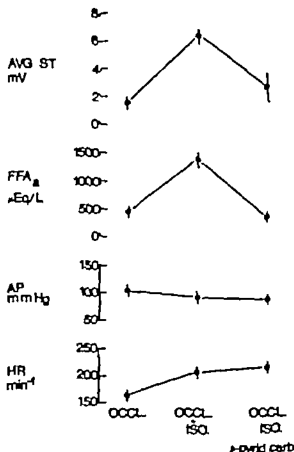


Fig 5 Effect of soproterenol on average epicardial ST-segment elevations and free fatty acid concentration befor and after pret tment with β pyridyl carbinol
AVG ST A rage epicardial ST-segment elevation
FFA Arterial plasma f ee fatty acid oncentration
AP Arterial pressure
HR Heart rate

ular pre sures nd left ventricular dP/dt wa unaffected by β pyridyl c rbinol (1) It wa also shown th t β pyrid l carbinol in the doses required for complete inh bition of lipolysi did not effect cha ges in ronary ulation or collateral coronary flow to the ischemi rea (1). More recently we comp ed the effect of gluc gon and isoproterenol on infar t ize 16) In equipotent inotropic doses gluc gon did not timulate lipolysis. Hence t wa f und that ST segment elevation after coronary occlusion w s large w th soproterenol than w th gluc gon however this disp rity in epicardial ST segment elev tion wa abol hed fter h bition f lipoly S milar e su ha e been btai ed in ou labor tory w th dop mine and c lcium De pite m ar not pp ffect the latter doe not timulate lipolytic act ty (17)

The effect of norepinephrine on the heart is more complex than that of isoproterenol Besides the increase in myocardial contractility and the stimulation of lipoly is coronary perfusion pres ure is also increased as well as myocardial wall tension. Norepinephrine therefore imposes increased oxygen requirement on the myocardium on the one hand and secures increased perfusion pressure on the other. Using ST-segment mapping we therefore studied the effect of norepinephrine given during coronary occlusion (18) and f und that norepinephrine incre sed the ischemic are However when the l polytic effect w s inhibited imilar admini tration of norepinephrine with imilar elevation of bl od pressure re ulted in a clear reduction of the ischemic area a well as ischemic changes in each partic epicardial site compared to control occlusion when no drug wa given (Fig 6). Obviously

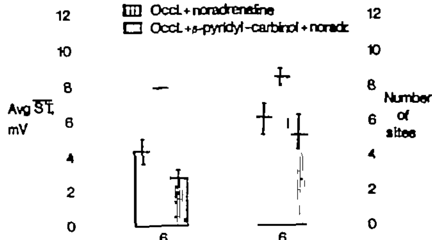


Fig. 6. Effects of norepinephrine before and during inhibition with β-pyridyl carbinol. Epicardial ST segment elevation and number of epicardial sites with ischemic electrocardiographic changes. Figures below columns indicate number of animals (18).

with norepinephrine alone the increase in oxygen requirement of the ischemic myocardium is larger than the increase in oxygen supply. After eliminating the lipolytic effect of norepinephrine the increase in oxygen supply overrides the remaining increase in oxygen requirement resulting from increases in mechanical activity. The effect of lipolytic inhibition is only temporary and the lipolytic activity returns to normal, or even super-normal levels 4 to 5 hours after a single dose of β-pyridyl carbinol. Repeated coronary occlusion at this time during noradrenaline infusion demonstrates the return of lipolytic stimulation and that this is associated with augmented ST-segment elevation (18)

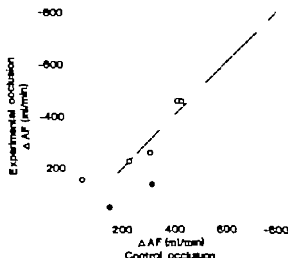


Fig. Reduction in cardiac output (ΔAF) during repeated coronary occlusion in two groups of animals. The first group (O) received no treatment, the second (●) received β-pyridyl carbinol intravenously started before and maintained during the second occlusion. The line of identity is indicated.

thus confirming the relationship between lipolytic activity and ischemic injury during acute coronary occlusion.

LIPOLYSIS AND ISCHEMIC HEART FAILURE

Catecholamine release takes place locally in the ischemic area (19) as well as from peripheral stores. The present study suggests that the enhanced lipolytic activity induced by catecholamines are detrimental to the evolving myocardial infarction and may therefore depress the cardiac pump function.

Conversely, salvage of ischemic myocardium might improve left ventricular function. The use of β-pyridyl carbinol during myocardial pump failure following acute coronary occlusion was therefore studied (20). Ischemic heart failure was induced by occluding the dominant one of the two major coronary arteries to the left ventricle. This procedure is followed by ischemia to an average 40 % of the left ventricle and by marked reduction in cardiac output. Interventions with β-pyridyl carbinol were found to counteract the reduction in cardiac output otherwise observed following acute coronary occlusion (Fig. 7). This suggests that the regression of ST-segment elevation obtained with antilipolytic drugs indicates salvage of myocardial functional capacity provided this treatment can be begun before irreversible damage occurs.

CLINICAL TRIAL WITH ANTI LIPOLYTIC TREATMENT

Preliminary trials in patients with acute coronary occlusion have demonstrated elevated free fatty acids during the first 4 hours after the onset of

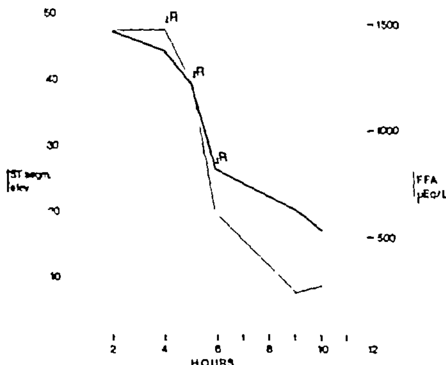


Fig. 8. Average precordial ST-segment elevation and arterial free fatty acids in a patient with anterior infarction treated with β-pyridyl carbinol 200 mg times three (R). The first dose was given 4 hours after the onset of symptoms. Average ST-segment changes were obtained from 34 precordial leads described by Maroko et al (1).

symptoms which can effectively be lowered to subnormal levels in less than hours during treatment with β-pyridyl carbinol. By using precordial mapping of ST segment changes as a quantitative estimate of the ischemic injury (1) it was found that reduction of plasma free fatty acid concentration was associated with parallel reduction of the ST segment changes (Fig. 8).

Inotropic and metabolic interventions during acute coronary occlusion have been effective in modifying infarct size. The release of free fatty acids induced by catecholamines seems to play an important role in the evolvement of acute ischemic injury of the myocardium. Inhibition of lipolysis may therefore confer considerable protection upon the myocardium. From a clinical point of view it is of particular interest that β-pyridyl carbinol does not interfere with the contractility of the nonischemic myocardium and can therefore be used without risk in patients with cardiac decompensation.

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DISCUSSION

Dr F t.gerald

How many of your dogs fibrillated in the experiment when you were tying the coronary artery

Dr Kjek hu

In these experiment on erage 30 per cent of all animal fibrillated.

Dr Fit.gerald

So you did not include those in the studies

Dr Kjekshus

No Only animals which did not fibrillate are included in these experiments.

Dr Fit.gerald

I am interested to know the relative efficacy of clofibrate in reducing free fatty acids as compared with the nicotinic acid.

Dr Mjøs

As you probably saw from the slide the efficiency of clofibrate in this respect is less than that of nicotinic acid With nicotinic acid you can completely block the rise in free fatty acids caused by isoprenaline but not with clofibrate but still the reduction I significant by 30-40 per cent with clofibrate This seems to be sufficient for a marked reduction of the severity of the ischemic injury since the main purpose is probably to reduce excess free fatty acids.

Dr Fitzgerald

Thus clofibrate and nicotinic acid ha e equal effect on the ST-segment but clofibrate has only 90 per cent of the effect of nicotinic acid in lowering free fatty acid

Dr Kjekshus

The arterial levels of free fatty acids can only indicate what is going on. You cannot use arterial concentrations s a quantitative index of what takes place in the infarct.

Dr Braunwald

Dr Kjekshus, could you enlarge on your clinical experience How many patients have you studied so far?

Dr Kjekshus

So far I have studied 14 patients with β-pyridyl carbinol and about the same number of patients without. There are two groups one receiving treatment and one not receiving any treatment other than oxygen and analgetics

Dr B aunwald

And what did you observe

Dr Kirk h s

It i the same trend I have conducted the treatment f r nly 10 hour ST segment are initally reduced the tre ted gr oup but when tre tment is discon tinued ST segment elev tion returns c ncomitantly w th elev tion of free fatty acid The treatment will n w be extended f r more than 4 hours

Dr Hjalmarson

Maybe we should go on to the general discussion and try to see if we can solve some questions together. I think one very important question that could be discussed first is where are the effects of fatty acids in the heart? Are they on the cellular membrane or on oxygen consumption. Is it possible that catecholamines together with high fatty acids change the cellular membranes getting more leakage of ions into and out of the cell.

Dr Mjøs

This is a very important question and I do not think I can add much to it. But in addition to the uncoupling effect of free fatty acids I would like to draw the attention to the recent work of Shrago Shug and Bittar in the U S. They demonstrated that long-chain fatty acyl-CoA esters which accumulate in the ischemic myocard, inhibit the translocation of adenine nucleotide transport across the mitochondrial membrane.

Dr Hjalmarson

I would like to hear Dr Neely comment upon this. If you force the heart to take up more fatty acids what changes will you see in contractility and metabolism of the heart.

Dr Neely

I would like to comment on the increase in oxygen consumption first since this has been mentioned several times. One can calculate that oxygen consumption should be about 15 per cent higher in hearts oxidizing fatty acids as opposed to carbohydrates. This is due to the production of FADH in β-oxidation which produces only two ATPs per oxygen used as opposed to three ATPs where NADH is oxidized. The levels of plasma fatty acid that caused increased oxygen consumption in the last two talks are high enough to suppress glucose utilization. It is likely therefore that these hearts were oxidizing mostly fatty acids and should have higher ratio of oxygen consumption. In the rat heart increasing fatty acid concentration to as much as 1.5 mM has little or no effect on contractility. The rat heart, however is very resistant to fibrillation during ischemia and it may be unfair to compare the effect of fatty acids in the rat heart to what may happen in other species. The accumulation of long chain acyl-CoA and the associated inhibition of adenine nucleotide translocation along with less efficient utilization of the residual oxygen available are important factors to consider in the development of ischemic damage. In connection with this, I would like to know if any one has measured plasma levels of fatty acids in whole animal models of ischemia and if a decrease in circulating fatty acids could account for the beneficial effects of glucose-insulin-potassium treatment?

Dr Mjøs

I am not giving glucose-insulin-potassium to try to reduce the size of the ischemic injury. Probably Dr Kjekshus has done it. But I have demonstrated in anaesthetized dogs that the rise in plasma concentration of free fatty acids effected by isoprenaline was markedly reduced by i.v. administration of 10 per cent glucose with a consequent fall in myocardial oxygen consumption. So the possibility is that part of the beneficial effect of glucose or glucose-insulin-potassium may be mediated through this mechanism.

Dr Bruyneel

We used in Cape Town with Dr Opie glucose-insulin-potassium in baboons and found a 50 per cent reduction of free fatty acids within the first hour of treatment of acute myocardial infarction. We did the same thing with patients in the coro-

nary care unit We were sampling free fatty acids and just by giving carbohydrate infusions or oral glucose such as tea with a high amount of glu cose we found a very sharp decrease of the circulating free fatty acid

Dr Hjalmarson

I think that sound very likely Does anyone think that one can force a heart to make itself more ische mic by utilizing more fatty acids I mean if you have a reduction in coronary flow that will give some degree of ischemia and then you force a heart to take up more fatty acids will that make the ischemia more severe? If there is a reduction in oxygen available in the cell that should result in a blockade of fatty acid oxidation and then if any thing there should be an accumulation of fatty acids in the heart cells A was suggested by Dr Neely this accumulation of intracellular fatty acids will interfere with the ATP production which is more l kely than just to make the oxygen ten ion of the heart cells lower

Dr Kjekshus

I do not think the possibility can be completely ruled out In an ischemic heart myocardial contractil ty c n be reduced to the same extent either by increa ing the upt ke of free f tty acid or by further reduction of O_2 delivery to the myocardium. How ever the amount of O_2 depri ation necessary to match the depres ion of contractility induced by free fatty acid corre pond closely to the rise in O_2 con mption induced by imilar uptake of free fat ty acid n the non schemic heart

Dr M ell

Dr Mjos you how enhanced myocardial glucose uptake in ne of you groups Do you know the mechanism Doe pyru te dehydrogenase play a med ating role The e son why I m particularly interested in you f nd ngs based on result in patient with acute myocardial infarction We mon tored my cardial metabolism over 7? hours in 40 patient with a te my c rdial infarction One group with enhanced my cardial glucose uptake had an n omplicated hos l ourse in contrast t the group with enhanced myocardial free fatty acid uptake demon t at g tendency to c r diac f lure nd arrhythmia

Dr Mjø

It would appear likely that the reduction in ische mic injury achieved with sodium dechloroacetate was related in part to an increased ATP production from anaerobic glycoly i in the ischemic cell sec ondary to a stimulation of phosphofructokinase In the less severely ischemic cells the oxidative me taboli m of glucose may also have been enhanced through an activation of pyruvate dehydrogenase The work of Opie and co-workers (Eur J Clin Invest 3 419 1973) ha shown that a considerable de gree of aerobic metabolism may persi t in some areas of the ischemic myocardium following coro nary occlusion in dogs. Measurements made on coronary inus blood during coronary occlusion may however conceal important differences be tween the metabolism of the ischemic and non ischemic zones.

Dr M ller

I would like to direct one question to the audience We know that catecholamines increasing lipolysis and arterial free fatty acids (FFA) content enhance FFA uptake by the myocardium Is there evidence *that catecholamines enhance also the utilization of* FFA in the mitochondrium

Dr Hjalmar on

It is surpri ing that so little is known about the control of the uptake and utilizat on of free fatty acid in the heart A number of studies ha e been performed on glucose uptake and on amino acid tran port

Dr Mjø

One comment I think it is important to bear in mind that one thing i myocardial upt ke of free fatty acid that I arterio-coronarovenous d fference of free fatty acid multiplied with myocardial plasma flow But the free f tty acids taken up by the heart are either oxidized or being esterified In the ische mic are esterification i enhanced relative to that of oxidation And the esterification of free f tty acids does also increase the oxygen demand of the ischemic zone although to a mall extent

D Speak

Much comme t has been made of the r le of f tty acid n the dise sed he rt and in p rticular whether

they might affect membrane function or oxygen consumption. A lot of measurements of fatty acids have been reported. Did these all refer to peripheral levels or can we consider the possibility of localized high levels of fatty acids occurring in the vicinity of the myocardium. In other words, is it possible that there is a high rate of release of fatty acids from triglyceride within the vicinity of the heart.

Dr Neely

Certainly lipolysis occurs in the heart but I do not know if this increases under ischemic conditions. There is enough fatty acids from lipolysis to cause a large increase in long-chain acyl-CoA in glucose-perfused hearts. Accumulation of long-chain acyl-CoA in specific areas on the mitochondria or in the mitochondria creates a very high concentration of these compounds in a small volume. Long-chain acyl carnitine accumulates in the cytosol and these compounds may bind to mitochondrial as well as other membranes.

Dr Gudbjarnason

There is certainly evidence that there is lipolysis in the myocardium. There are technical difficulties in estimating this and one way to get around this is to look at the release of glycerol. Infusion of isoproterenol increases the AV-difference of glycerol, that is a release of glycerol is being observed. It is important to consider the various effects that the fatty acids could have. There is great deal of interest in the transformations and transacylations in various structural lipids. Elevation of the free fatty acid level may affect structural lipids. The electro-chemistry of the membrane is quite important and the electro-chemical effects of these free fatty acids should be studied. So far the interests are concentrated on the role of the fatty acids as a substrate but they have also an important role to play in structural lipids.

Dr Mjøs

There is a study by Christian and co-workers (1969) giving isoprenaline to the isolated rat heart. Isoprenaline greatly increased the efflux of glycerol from the heart. Pretreatment with nicotinic acid in the appropriate dose markedly reduced the isoprenaline induced efflux of glycerol from the heart. In preliminary experiment we have recently demonstrated similar findings with clofibrate (de Deckere, Mjøs and Miller, unpublished observations).

Dr Hjalmarson

Before we leave this, I think it would be interesting to hear whether there is any need for nicotinic acid or clofibrate to reduce free fatty acid levels if we have beta-blockers. If we have a good beta-blockade that will inhibit heart function and reduce free fatty acid levels – can you add something extra to that by nicotinic acid or clofibrate or some other blocker of lipolysis. We are well aware that at least some of these substances reducing free fatty acids will give some problems for the patients. Of course beta-blockers could do that too. It could be interesting to hear, especially from Drs Mjøs and Kjekshus, if you believe that it is enough to have one substance or if in the clinical practice we should combine a beta-blocker and a substance reducing free fatty acid levels.

Dr Kjekshus

Beta-blockers will probably interfere with catecholamine dependent lipolysis and I believe if you should make a choice, you should probably use beta-blockers on patients with very small infarctions as long as the hemodynamic situation can be controlled. β-pyridyl carbinol (Ronicol) should be preferred in patients with pump failure because β-pyridyl carbinol does not depress myocardial function.

Dr Hjalmarson

Is there no discomfort for the patient to take these. I think you need a high concentration, do you not.

Dr Kjekshus

You need a high concentration of β-pyridyl carbinol in order to reduce the free fatty acids to subnormal levels. Most patients will have a flushing in their faces.

Dr Hjalmarson

Having an acute myocardial infarction and feeling ill – would you like to add side-effects of a drug like Ronicol. You have been working with that on patients and it could be very interesting to hear if they have had any problems.

Dr Kjekshus

The problem with the flushing is transient. It is gone after one hour despite continued infusion of

β-pyridyl carbinol. If it reduces their infarct size I think it is very easy to convince them.

D Hjalmarson

Yes if there is no other way to reduce the infarct size I agree

Dr Fitzgerald

It seems probable that clofibrate and Ronicol are acting at different sites when inhibiting lipolysis Do you have any views on combining them I have one methodological comment. Are we wise to use isoprenaline as the sole agonist in our experiments Would it not be more relevant to use either noradrenaline or adrenaline in the routine use of an agent to raise the ST-segments The constant use of isoprenaline conceivably could mislead us interpreting the results.

Dr Mjo

Qualitatively I think that the FFA-lowering effect of clofibrate and nicotinic acid is the same but quantitatively different. Clofibrate may have less side effects than nicotinic acid. But at the present time the drug of choice to a patient in the acute stage of myocardial infarction is ery difficult to assess A combination of clofibrate and nicotinic acid would most likely have similar beneficial effects as each of the drugs given separately Preliminary data from Dr Oliver s group in Edinburgh suggest that a nicotinic acid analogue with less side effects than plain nicotinic acid, given *per os* reduced ST-segment elevation in patients with acute myocardial infarction provided early treatment was initiated. Regarding clofibrate I am very much interested in that ICI would clear an i v form of clofibrate for human use because I think that the FFA-lowering effect of clofibrate given *per os* is not so quick and marked as wanted

Dr Hjalmarson

I am afraid that I did not make myself clear I never thought of combining clofibrate and nicotinic acid I thought of having beta-blockade and nicotinic acid or clofibrate

Dr Braunwald

What happens to the FFA-level in patient who have been taking clofibrate who develop a myocardial infarction There must be many such events.

Dr Mjø

As far as I know if people on long-term treatment with clofibrate get an acute myocardial infarction, the drug is immediately withdrawn, because hitherto clofibrate has been considered as potentially dangerous in this situation. The idea has been that there is a competition between clofibrate and free fatty acids for binding sites on the albumin molecules so that clofibrate might divert free fatty acids into the tissues with a potential harmful effect on the ischemic injury However our view is that the amount of albumin in plasma is sufficient for binding of both clofibrate and free fatty acids in plasma, and moreover that clofibrate reduces myocardial uptake of free fatty acids with a beneficial effect on the ischemic injury

Dr B aunwald

When a patient who has been receiving clofibrate chronically is given an infusion of isoprenaline – is the FFA rise affected

Dr Mjøs

From the work that has been done by others in humans a short treatment with a daily dose of g clofibrate is sufficient for a significant FFA-lowering effect. But I do not know whether chronic treatment with clofibrate for 5 or 10 years would be effective in the inhibition of isoprenaline-induced lipolysis.

Dr Hjalmarson

I think that there must be some data from ICI on this topic Maybe Dr Fitzgerald can comment upon that. If you use clofibrate for a very long time, will that reduce the isoprenaline-induced increase in fatty acid levels

Dr Fitzgerald

I cannot answer this question.

Dr Hjalmarson

That will end the first part of the afternoon session. I would like to thank the participants for presentations and discussions

SOME TRENDS OF THE NATURAL DEFENSE AGAINST THE CARDIAC ANOXIA

O Poupa

Department of Clinical Physiology
University of Göteborg, Sweden

INTRODUCTION

The present Symposium deals with problems concerning protection of the heart muscle against ischemia. The aim of my report is to focus the attention to some vertebrates which either spend their life in the habitats where the O_2-availability is poor or their mode of life puts them periodically under hypoxia of considerable degree and duration (hibernators, estivators) During the past decade the evidence was accumulated, showing that hearts of these animals tolerate rather high degree of acute O_2-lack (4 7 8 14 17 19 20) and some of them show also considerable immunity against necrogenic stimuli (18 19). Let us summarize these data and let us try to pick up some natural trends of defense by which the functional integrity of the cardiac cell is preserved when subjected to the acute lack of oxygen.

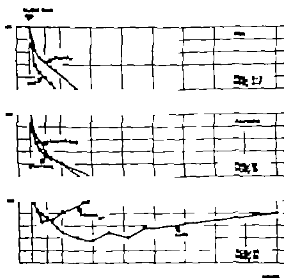


Fig 1 Comparison of the cardiac force decay by anoxia in different poikilotherm vertebrates.
Isometric ventricle strips. Pacing 12/min 12°C L_{max} 97 % O_2 + 3 % CO_2 Ringer with 11.9 mM $NaHCO_3$ NaCN 3 mM (arrow). Species tested: *P platessa G morrhua R pipiens R temporaria, P scripta Varanus.* Ordinate: cardiac force expressed in % of preanoxic values. Abscissa, time in hours. Figures at the right side of the plots, mean physiological PCO_2 and HCO_3 in the blood of the respective class of animals according to (25) and (5)

ANOXIC TOLERANCE OF THE HEART MUSCLE IN DIFFERENT VERTEBRATES

It is generally accepted – although as concerns underlying mechanisms not fully understood – that the heart muscle of the poikilotherms is less susceptible to the anoxic damage than the heart muscle of the homoiotherms (16) But within these two groups of vertebrates considerable differences in the cardiac anoxic tolerance exist.

Poikilotherms Fig. 1 shows differences between three main poikilotherm classes – fish, amphibia and reptiles – when the heart muscle strips are subjected to the sudden block of the cellular respiratory pathways by a standard dose of cyanide under comparable conditions as concerns cardiac work (pacing) temperature and acid-base balance of the tissue. Hearts of the fish and amphibia reduce the force very rapidly and within approximately one hour they stop to work. In contrast, the heart of some reptiles regains after initial loss of the force the preanoxic work performance showing perfect spontaneous reversibility of the function, only temporarily depressed by the lack of energy derived from the respiratory metabolic pathways.

Fig. 2 gives a more detailed insight into the situation among different species of fish (11 species total n of animals. 91) When the heart of the Atlantic cod (*Gadus morrhua* n. 19) and the heart of the flatfish (*Pleuronectes platessa* n: 15) are forced to work under a complete block of the cellular respiratory pathways in otherwise similar conditions, the latter will reduce the force to the half of the preanoxic value within 45 min the former within 4 min

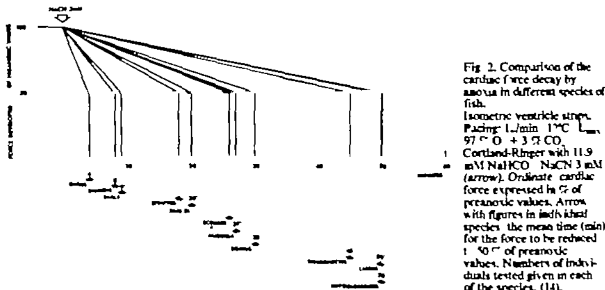


Fig. 2. Comparison of the cardiac force decay by anoxia in different species of fish.
Isometric ventricle strips. Pacing: 1./min 17°C L_{max}, 97 % O_2 + 3 % CO_2, Cortland-Ringer with 11.9 mM $NaHCO_3$. NaCN 3 mM (*arrow*). *Ordinate*: cardiac force expressed in % of preanoxic values. Arrows with figures in individual species: the mean time (min) for the force to be reduced to 50 % of preanoxic values. Numbers of individuals tested given in each of the species. (14).

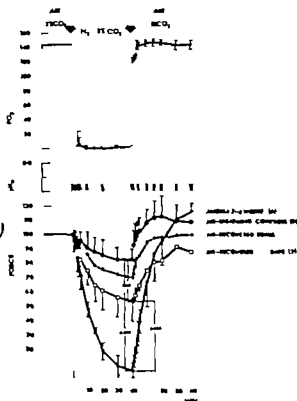


Fig. 3. The effect of the long-term systemic anoxia on the cardiac muscle of the turtle (*Chrysemys picta elegans*). Upper tracing: PO_2 in the Ringer. Middle tracing pH of the Ringer. Lower tracing: cardiac force in % of preanoxic values. Isometric ventricle strips (pacing, 12/min 12°C L_{max}). Abscissa: *time in min. Numbers in bracket*: number of animals in respective experimental groups ± SD. See text. (22).

The ratio between the least and the most tolerant species was 1:1 (4).

As shown by Fig. 1 among all poikilotherms the heart of reptiles seems to be best equipped for the survival in anoxia and above all of them the heart of the turtle deserves attention. Experiments given by Fig. 3 illustrate these exceptional properties. Turtles (*Chrysemys picta*) were placed in a complete anaerobiosis (N_2-saturated water – +6°C) for 2-6 weeks. After this period the heart was still alive. However under the acute O_2-lack *in vitro* it reduced the force more than the heart of control air-breathing animals but the recovery after reoxygenation was complete. When the anoxic animals were allowed to return to the normal atmosphere the reaction of the isolated heart muscle to the acute anoxia was within three days identical with control as a sign of the regained integrity (23).

Homoiotherms. A more extensive study comprising different mammalian species as concerns resistance of their hearts to anoxia is lacking. Nevertheless, two types of the mammalian muscle were found showing exceptional tolerance to the anoxia: hearts of animals adapted to high altitude (2, 8, 14, 17, 19) and hearts of some hibernating rodents (2, 20). Under low PO_2 a slower decline of the cardiac force was registered in high-altitude adapted rats in comparison with sea-level controls (2, 14) and an analogous situation was observed in isolated diaphragms (7). Hearts in two species of hibernating rodents behaved in a similar way (*Glis glis*) 20, ground-squirrels 7). Accordingly better force-recovery during reoxygenation was registered in both high-altitude adapted rats and hibernators

(8, 17 19 20) A considerable resistance of the myocardium against necrogenic stimuli was shown in rats adapted to high-altitude hypoxia (18, 19).

From all these data a conclusion can be drawn that among vertebrates some species can be found in which the heart shows rather high anoxic tolerance which is not connected with poikilothermia, among poikilotherms some fish and some reptiles among homoiotherm animals living in high altitude and hibernators. Habitats in which these animals can be found are characterized by low O_2-tension (muddy ground high terrestrial elevations etc.) or they concern also a special mode of life (hibernation estivation).

SOME UNDERLYING PHYSIOLOGICAL MECHANISMS

Information concerning mechanisms which are connected with high tolerance of the heart muscle to the acute O_2-lack is far to be complete. Nevertheless, some data were collected and some general patterns of the natural defense of the heart muscle against the anoxic stress can be traced. The main physiological mechanism represents the reduction of the cardiac work under the anoxic period. Bradycardia, sometimes of a considerable degree during the exposure to the anoxia, is the main feature during hibernation estivation and diving. The reduced circulating blood-volume is preferentially drained to the brain, the prototype of such blood-distribution being well known since long in diving mammals (6) The second mechanism is the drop of the body temperature Lowered cardiac work-load and cooling can essentially contribute to the preservation of the functional integrity of the mammalian heart muscle under acute anoxia, as illustrated by Fig. 4 under constant anoxic stress the best preserved ability to regain the force by the reoxygenation was found in least working and cooled heart muscle specimens (9). It is to be noted that even the morphological cardiac lesions and mortality of the animals in which experimental cardiac necroses were induced by isoproterenol, were enhanced by increased body temperature and this effect was neutralized by all interventions enhancing heat release during the critical period (3 21).

Let us now deal with a third essential factor contributing to the survival of the cardiac cell during anaerobiosis. the exchange of H-ions between the cardiac cell and the extracellular space. Keeping in mind the construction of metabolic pathways mainly used during cardiac anaerobiosis, the excess of protones is obvious. Under unlimited perfusion (hypoxia or anoxia) the release of H is free, although even under these conditions lowered intracellular pH was recently reported (10) indicating that the H-ion production exceeds the sum of both release and the buffering capacity of the cell. The situation became however far more critical under ischemia (1 15). Fig. 5 shows that the development of the contractile force under failing cellular respiration largely depends on how much H is allowed to leave the intracellular space first beats reduced by sudden respiratory failure seem to be pH independent but the amount of the force developed in the long run under limited ATP production is largely determined by the extent of H-ions release from the cardiac cell (24). Identical result was obtained when the ATP production was reduced by the O_2-lack. the force decline was greater when the release of H was inhibited (Fig. 6). But even greater effect was registered during recovery by the reoxygenation. the slope of the plot expressing the relation between extracellular pH and force decay was 11 1 ± 0 95 % F/pH unit and force recovery was 32.7 ± 0.85 % F/pH unit (see Fig. 7) It is to be stressed that the effect of the limited H -exchange on the cardiac contractility became more apparent with the increasing severity of the anoxic stress until the cellular respiration was intact or very slight

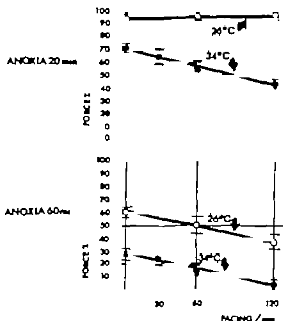


Fig. 4 The effects of the cardiac work and temperature on the force recovery by reoxygenation after N_2-anoxia of different duration.
Male rats. Right ventricle isometric strips (Krebs-Ringer pH 7 3 L_{max}). Abscissa. pacing/min. Ordinate: % of force regained by reoxygenation after anoxia (N_2 20 and/or 60 min) at 26 or 34°C ± SD (9).

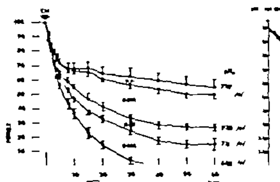


Fig. 5 The effect of the H-ion release on the cardiac force developed under acute cellular respiratory failure by cyanide
Frog (*R. pipiens*) ventricle strips. Pacing: 12/min, 12°C; 75 % L_{max}, 97 % O_2 + 3 % CO_2. Ringer containing 35.7 (pH 7.77), 23.8 (pH 7.56) 11.9 (pH 7.33) 5.9 (pH 7.0) or no (pH 6.02) mM $NaHCO_3$. NaCN 3 mM (arrow).
Abscissas: time in min. Ordinates: force in % of preanoxic values (left plot) and in the log scale (right plot). Numbers in brackets: n of experiments in the respective group (24).

ly depressed the effect of H was small but became more expressed as the block of respiratory path ways increased (Fig. 8) or as the duration of the O_2-lack was prolonged (Fig. 9). Interrelations between PO_2, release or retention of H and force development during both decay or recovery period are plotted on Fig. 10. As the PO_2 decreases the force declines. However the final loss of the force at the constant low PO_2 (≐ 7 torr) is largely dependent on the extent of H -release. The recovery by reoxygenation (broken lines) is complete when the force loss does not exceed 50-60 % of preanoxic values but did not take place at all when the force during anoxia declined under 10 % of the preanoxic values. The chain of events, e.g. PO_2 decline – insufficient H -release – force decline – force recovery by reoxygenation is evident, but the causative sequence of individual members of this chain remains to be elucidated.

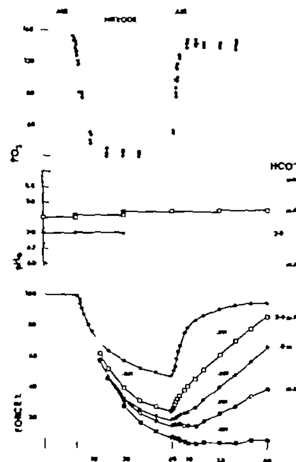


Fig. 6. The effect of the H-ions release on the cardiac force decay and recovery after N_2-temporary anoxia
Frog (*R. pipiens*) ventricle isometric strips. Pacing: 12/min 12°C 75 % L_{max}. Upper tracing: PO_2. Middle tracing: pH resp. mM $NaHCO_3$ of the Ringer. Lower tracing: force in % of preanoxic values. Abscissa: time in min. (25).

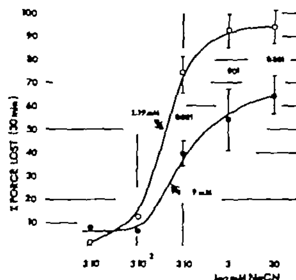


Fig. 7 Relations between force decay (black points heavy line) under N_2 – temporary anoxia, force recovery after reoxygenation (open circles – broken line) and extracellular pH (pH_e – abscissa). Ordinate: relative force in 30th min of decay and/or recovery. Replotted from Fig. 6. (25).

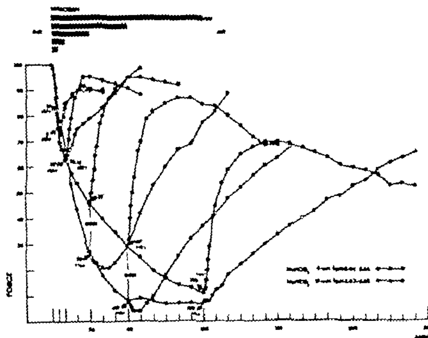


Fig. 8. Effects of the H-ions release on the loss of the cardiac force during cellular respiratory failure of different degree
Frog (*R. pipiens*) ventricle isometric strips. Pacing: 12/min 17°C 75 % L_{max} 97 % O_2 + 3 % CO. Ringer contained either 1.19 (open circles) or 11.9 (black points) mM $NaHCO_3$. Abscissa, log mM NaCN. Ordinate: % of force lost within 30 min after NaCN

The question arises, whether the cardiac cell depressed by accumulated protones during temporary anaerobiosis is dead or living. Experiments summarized in Fig. 11 on the paired samples of the myocardium seem to indicate that the functional integrity could be regained when the release of the protones was resumed. The similarity between the behaviour of the ischemic cardiac cell and fatigued skeletal muscle (1., 13) deserves attention in many respects.

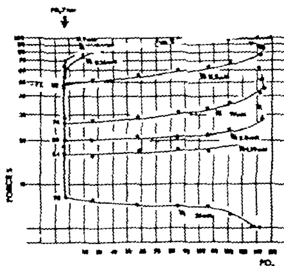


Fig. 9. Relations between the duration of the N_2-anoxia, cardiac force decay and/or recovery after reoxygenation and H-ion release
Frog (*R. pipiens*) ventricle isometric strips. Pacing: 12/min, 17°C 75 % L_{max}. Black bars with numbers: duration of anoxia in min. Black points: 11.9 mM NaHCO and 1.19 mM NaHCO (open circles) in the Ringer. Abscissa: time in min. Ordinate: force in % of preanoxic values. Numbers in bracket: of experiments.

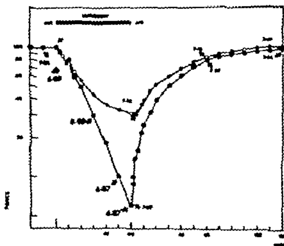


Fig. 10. Interrelations between PO_2 (abscissa), release and/or retention of H-ions and cardiac force (ordinate) developed under anoxia (full lines) or recovery (broken lines).
Frog (*R. pipiens*) isometric strips. Pacing: 12/min 12°C 75 % L_{max}. Ringer with 0.36-11.9 mM $NaHCO_3$ – see numbers with arrows pointing to respective broken lines. Ordinate: force-loss in % of preanoxic values (log scale).

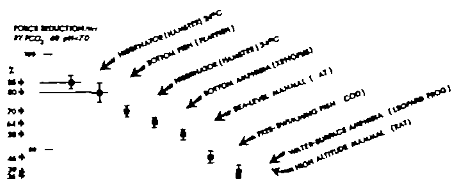


Fig. 15 Cardiac force reduction by raised PCO_2 (> 60 torr pH ≐ 6.9) of isometric heart muscle strips (ordinate: force in % of prenormic values 30 min after PCO_2 increase) Isolated from *animals living in hypercapnic* (hibernators, bottom-fish and amphibia), normocapnic and hypocapnic (high-altitude) conditions. Data compiled from (22) and by permission from (27)

exist except in one: in hearts adapted to the high-altitude hypoxia where the tolerance to the O_2-lack is high but the tolerance to the respiratory acidosis is low

CONCLUSIONS

Among vertebrates some exceptional species can be found which are able to preserve the functional integrity of their heart muscle under very low O_2-supply. They live under conditions in which not only the availability of the oxygen is poor but also the acid-base balance of their bodies is reshaped due to the modified ventilation.

Three physiological mechanisms seem to be involved in preservation of the cardiac cell during the oxygen lack. 1 depression of the cardiac work and 2 body cooling; in some cases 3 effective mechanisms cope with the H-ions produced in the excess by anaerobic myocardium. However exceptions from this general pattern exist (high-altitude animals) showing that some – not yet understood – mechanisms are involved which ask for further elucidation. There is no doubt about the effectiveness of the natural trends of defense against cardiac anoxia. They deserve attention because we can take a lesson from the wisdom of the body when treating ischemic myocardium in the medical practice

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DISCUSSION

Dr Kjekshus

We have been interested in studying animals which naturally adapt to anoxia in order to learn more about normal mechanisms for myocardial adjustment to anoxia. We have therefore studied the diving seal and I would like to convey to you some of the results During the dive heart rate is reduced to 10 beats *per minute from 122 124 beats per minute* Arterial perfusion pressure is maintained almost unchanged in spite of the reduction in heart rate Myocardial contractility is reduced by 25 per cent and coronary flow is reduced to 10 per cent of control values i.e 10 *ml per minute per gram* tissue which is an astounding reduction. The preload or the venous return to heart is markedly reduced and cardiac output amounts to 10 per cent of control value The arterial levels of glucose are increased while FFA concentrations are reduced. If we could mimic these changes in patients with acute coronary occlusion we should probably be able to prevent ischemic injury

Dr Poupa

Do you have some data concerning the buffering capacity of the heart muscle in rats How much will the blood pH change by diving

Dr Kjekshus

The pH in arterial blood was very little changed.

Dr Braunwald

There are a number of circumstances in man in which these observations become important. First the normal fetal heart is exposed to a much lower PO_2, than postnatally, and I am interested in whether you have any information about how much energy it is capable of receiving from glucose. Secondly, many patients with cyanotic congenital heart disease have adequate cardiac function despite the fact that they never reach a normal arterial PO_2. Is there some enzyme induction that takes place in these patients or is there an enzyme adaptation that normally takes place at the time of birth

Dr Poupa

To the first question about the fetal heart. Fetus is really a diver and inhabitant of high altitude and its heart is adapted to these conditions. As concerns energy taken from the glucose, I think that this can be supposed, but if such data exist they can be found in papers by Dr Friedman who has studied this problem. He explains differences between fetal and adult hearts from the point of the development of the sympathetic innervation in transition from the fetal life to the adult life. There are some findings in Sweden concerning low sympathetic innervation in hibernators which are also showing high resistance to anoxia. But all this is extrapolations only, experiments are quite new and we need more information as concerns the cyanotics. It has been shown that the heart of cyanotics contains LDH containing more M subunits. To my knowledge this is the only information available. When rats are grown from the weaning on the high altitude, they are able to preserve fetal type of LDH (more M units). But I do not think that LDH-M is the main system which is involved substantially in those mechanisms which make the heart of high-altitude animals more resistant to acute anoxia. Our recent comparative studies on hearts with quite a large spectrum of H and M LDH unit combinations substantiate this conclusion.

FACTORS MODIFYING ISCHEMIC ALTERATIONS OF VENTRICULAR FUNCTION AND METABOLISM IN THE INTACT WORKING SWINE HEART

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In the past several years important new insights have been made concerning the metabolic changes which occur with myocardial ischemia (1 9). However the interrelationships of these changes which result in a loss of metabolic homeostasis and the development of abnormal mechanical function, remain imperfectly understood Answers to these questions are currently being explored in many clinical and research laboratories but at present are procedurally limited by several inherent restraints in protocol design and/or experimental models While most new data have been obtained in a variety of animal preparations including *in vitro* myocardial strips slices homogenates and isolated *perfused hearts of rat, guinea pig, rabbit* and dog (10-13) the worry persists that the findings may be remote oversimplified and/or inapplicable to the ischemic processes in man. Patient studies have likewise been limited in the kinds of data which can be collected and are presently confined to analyses made either at operation or cardiac *catheterization* Such restrictions in collections and interpretations of data have renewed the search for animal models of ischemia which more closely approximate those conditions present in man.

Perfused heart preparations offer many advantages for metabolic studies including an intact delivery of oxygen and substrates to the myocardial cell. Many current models however are isolated denervated hemodynamically uncoupled, and thus removed from the usual humoral and reflex influences existent clinically Moreover the perfusates used in these preparations have usually consisted of buffered salt solutions free of hemoglobin and other formed elements of whole blood and are gassed with oxygen mixtures at partial pressures much higher than physiological levels A new myocardial ischemic model was developed in this laboratory using swine with capabilities of controlling and regulating total coronary blood flow in an intact, *in situ* blood perfused working heart. The following is a brief description of this model system with some observations on the changes and distortions in mechanical and metabolic functions induced by restrictions in coronary flow and a report of one metabolic intervention with potential therapeutic efficacy

WORKING SWINE HEART MODEL

Swine were chosen for use in these studies because of their close proximity to man in cardiovascular function and anatomy (14) and coronary perfusion. Porcine coronary arteries are almost identical to man (15) in distribution, intramyocardial collateral formation and propensity for developing spontaneous atherosclerosis. Similarity of pharmacological esponsiveness to several cardioactive drugs has also been r ported (16-18). The preparation consisted of a right heart bypass in anesthetized, open-chested swine with extracorporeal perfusion assistance to maintain left ventricular pressure development *[and thus myocardial oxygen demand]* constant A second closed-loop extracorporeal circuit was constructed to perfuse the coronary arteries Reoxygenated venous blood from the right ventricle was returned to the separately cannulated left and right coronary arteries through an oxygenator/heat exchanger in series with two perfusion pumps separately controlling flow to each coronary artery Coronary perfusate consisted of the animal's own whole blood supplemented with additional insulin, glucose and fatty acid. All hearts were paced slightly in excess of their intrinsic rhythms

Various hemodynamic and metabolic measurements were made at 5 or 10 minute intervals throughout the course of coronary perfusion. A description of these methods are found elsewhere (19). Measurement of total coronary flow under physiological conditions was permitted in this preparation by simply diverting and quantitating the effluent drainage from the right ventricular pump over a short period of time prior to cannulating the

coronary arteries. In all ischemic studies total cor onary blood flow was initially held at normal physiological levels for at least 20 minutes of perfusion to allow for equilibration of substrates and hor mones between the central coronary reperfusion loop and the myocardium. Incremental rather than precipitous reductions in coronary flow were imposed on hearts to avoid difficulties with electrical instability leading to ventricular fibrillation. Coronary flow to each coronary artery was re duced in parallel to produce global or whole heart ischemia and ischemic perfusion was continued for a maximum of 30 minutes in most cases At normal coronary flow rates the preparation had stable hemodynamic and metabolic performance for at least one hour (20)

MYOCARDIAL ISCHEMIA

Initial studies with this model involved characterization of the changes in mechanical and metabolic functions which occurred with modulations in cor onary flow (Tables 1 and 2) With a 50 percent reduction in coronary flow (186.4 ± 5 6 to 93 1 ± 4 3 ml/min) mechanical performance remained in tact yet significant declines in myocardial oxygen consumption and uptake of glucose and fatty acids were noted The depression in glucose uptake occurred despite adequate insulin (0.025 units/ml perfusate) and most likely reflected inhibition of glycolysis which has been shown to result from prolonged exposure to ischemia (21) Decreased fatty acid uptake was observed but rates of fatty acid oxidation remained at control values Although somewhat restricted oxygen delivery in this group of hearts was apparently adequate to maintain mitochondrial function as evidenced by the rates of fatty acid oxidation and tissue stores of high energy phosphates resulting from oxidative phosphorylation. Glycogen utilization was increased as indicated by the decreased tissue glycogen (Table ?) and may be explained either by an early acceleration in glycolysis (5 1) and/or conversion of glycogen phosphorylase *b* to *a* as noted in other ischemic models (22) Tissue lactate was not increased

Yet more striking changes occurred with further decreases in coronary flow (183 8 ± 5.2 to 73 5 ± 4 ml/min Tables 1 and 2 60 per cent ischemic data) Mechanical performance was markedly impaired as indicated by an impressive rise in left ventricular end-diastolic pressures Ventricular dysrhythmias and ventricular fibrillation developed which significantly shortened the group sur vival time Further decreases in oxygen consumption and glucose uptake were also observed and mitochondrial function was clearly reduced Lack of oxygen diminished flux through the citric acid cycle as reflected by a 60 % reduction in $^{14}CO_2$ production from labeled palmitate Intracellular lactate accumulated and tissue stores of creatine phosphate and ATP decreased Tissue levels of ADP and AMP both increased and gly cogen utilization was further accelerated

Table 1 Hemodynamic and metabolic changes with varying ischemia.

Condition	Survival Time	Hemodynamics		Metabolics			
	min	PTM mm Hg sec/min	LVEDP mm Hg	MVO_2 μmol/hr/g	Glucose uptake μmol/hr/g	FFA uptake μmol/hr/g	FFA oxidation μmol FFA/hr/g
Control	60 0	2328	7 9	650	174 6	30 9	12.7
= 13		±187	± 9	± 70	±15 3	±3 5	±1 9
50 % Ischemia	40 0	2091	11 1	480 †	84 7 †	17 6 †	13 0
= 9		±13	±2 1	±50	±11 0	±2 7	±2.2
60 % Ischemia	29 8	2103	32.8 †	400 †	21 1 †	17 1 †	5 1 †
n = 10	±3 0	±328	±4 5	±10	±4 3	±4 5	± 3
Hypoxemia	49 9	2240	41 0 †	370 †	243 1 †	19 2 †	7 4 †
= 13	3 1	±342	±6 5	±10	±23 3	±3 3	± 7

Abbreviations PTM pressure time/minute LVEDP = left ventricular end-diastolic pressure MVO = myocardial xygen consumption = number of wine heart in each group † indicates statistical differences from control data with probability values < 05

Data are listed as mean alues ± one standard error of the mean (SEM) and expressed per gram dry weight of myocardium Control perfusion wer terminated after 60 minutes

Survival times for ischemic heart wer corrected for the initial 20-minut period at normal coronary flows

Perfusate concentrations of insulin 025 units/ml) glucose (15 mM) and fatty acid (4 mM) were comparable for all groups

Table _. Tissue stores of metabolites.

Condition	Creatine P µmol/g	ATP µmol/g	ADP µmol/g	AMP µmol/g	Glycogen µmol/g	Lactate µmol/g
Control	36.9 ±1.5	19.6 ± 3	2.31 ± 13	11 ± 01	252.0 ±13 9	16.9 ±2.5
50 % Ischemia	38.2 ±2.4	19 9 ± 7	2.53 ± 11	11 ±.04	199 9 † ±9 1	18.9 ±3 0
60 % Ischemia	11 4 † ±[illegible]	14 4 † ±1.5	3.89 † ± 27	78 † ± 10	143.0 † ±18 7	82._ † ±9.4
Hypoxemia	23 5 † ±5 0	15.3 † ± 9	2.91 † ±_4	11 ± 02	130 1 † ±6.9	65.8 † ±11.8

Abbreviations: P = phosphate ATP = adenosine triphosphate ADP = adenosine diphosphate AMP = adenosine monophosphate † indicates statistical differences from control data with probability values < 05 Data ar again listed as mean values ± one SEM

The critical role of coronary flow in preserving hypoxic myocardium was further underscored by the contrasting effects of hypoxemia (23) and ischemia (Tables 1 and ?). In the hypoxemic group of hearts coronary perfusion was maintained at physiological levels (191.5 ± 9 ml/min) but the perfusate was gassed with a mixture of N O_2:CO (90:5 5 %) Partial pressures of oxygen were about 30 mm Hg which resulted in a reduction in oxygen delivery and myocardial oxygen consumption to levels comparable to those produced with a 60 per cent restriction in coronary flow Although ventricular function deteriorated, hypoxemic hearts had less dysrhythmias and prolonged survival Tissue stores of creatine phosphate and ATP w re higher and intracellular accumulations of ADP AMP and lactate were less Both uptake and oxidation of fatty acids were increased and glycolysis was accelerated As first reported by Rovetto *et al* in the isolated perfused rat heart (?1) carbohydrate utilization varies markedly during oxygen deprivation depending on the additional influence of coronary flow In ischemic hearts there is absolute suppression of glucose metabolism while anoxic heart glycogenolysis and glucose uptake may be increased 10 to ?0 fold (24). Such differences probably result from stimulation of phosphofructokinase in anoxic hearts and inhibition of glycolytic enzymes n the glycolytic pathway principally at glyceraldehyde 3-P dehydrogenase (4) in ischemic hearts It appears that ischemia estrict these enzymes due to greater intracellular levels of H NADH and/or lactate Studies in the blood perfused swine heart demonstrated the crucial ole of coronary blood flow in preventing the intracellular build-up of inhibitory metabolic products The reported successes of coronary evascularization procedures in the emergency treatment of ischemic heart disease may be in part explained by these washout effects

METABOLIC THERAPIES

It is perhaps true that over the past decade or so more investigative effort has been expended in the search for a universally successful treatment of ischemic heart disease than in any other single area of adult cardiology Most early forms of treatment were based on bedside clinical empiricisms and consequently were limited by a lack of under standing concerning the various regulatory controls governing oxygen availability in the ischemic myocardium Recently the tenuous nature of the balance/imbalance between oxygen supply and demand in ischemic heart muscle was defined in the detailed animal studies of Maroko Braunwald, and others (25-29) These data suggested that myocardial oxygen needs are critically dependent on several hemodynamic parameters including heart rate contractility and afterload Appropriate integrated manipulations of these factors clearly reduced experimental infarct size in animals and have provided a new approach to the clinical treatment of patients with coronary artery disease and acute myocardial infarction.

In addition to reducing oxygen requirements via alterations in mechanical performance other treatments us ng different modalities to salvage ischemic myocardium ar currently being tested. Two such agents include hyaluronidase which is thought to increase extracellular diffusion and capillary permeability in ischemic tissue (30-32) and steroids which may inhibit the activity of myocardial lysosomes and stabilize phagocytic vacuoles of mobilized inflammatory cells (33-35). Another group of potential therapies designed to protect the ischemic heart have attempted to improve energy production through changes in metabolic pathways chiefly by efforts to accelerate carbohydrate metabolism. Early reports by Sodi

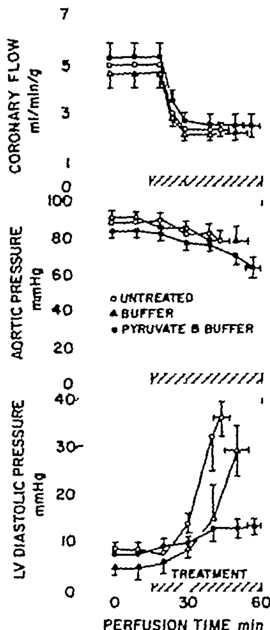


Fig. 1 Hemodynamic data in three groups of ischemic heart (O symbols denote the untreated hearts, ● indicates those hearts receiving (40 mM) pyruvate plus TRIS, and Δ represents hearts receiving buffered TRIS (25 mM) Coronary perfusate concentrations of insulin (.025 units/ml) glucose (25 mM) and free fatty acids (.7 mM) were comparable between groups. All heart were similarly restricted with regard to reductions in total coronary flow however only the pyruvate plus TRIS treated group had preservation in mechanical function and significantly longer group survival time. Bars on each data point encompass ± SEM

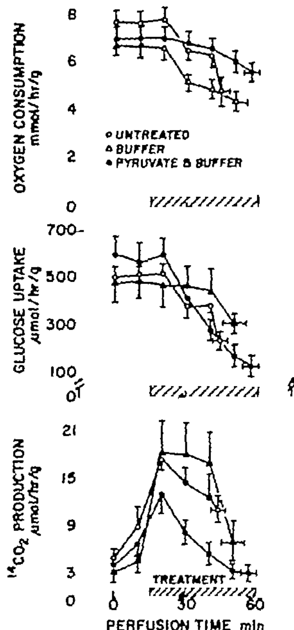


Fig. 2. Metabolic data in the same three groups of hearts. Myocardial oxygen consumption was slightly higher and fatty acid oxidation (estimated from the production rates of $^{14}CO_2$ from labeled palmitate) was significantly lower in the pyruvate plus TRIS treated group. Responses in the group receiving TRIS alone were not appreciably different from those in the untreated group

Pallares (36, 37) suggested that "polarizing solution" consisting of glucose insulin and potassium was beneficial in protecting ischemic myocardium. The solution was designed to replace intracellular potassium lost during myocardial ischemia and to provide additional glucose for anaerobic glycolysis. Initial results were hopeful in terms of normalizing mechanical electrical and metabolic functions in patients with coronary insufficiency and/or acute myocardial infarction (38-40) but

have since proved somewhat difficult to reproduce (9 41 42). In the working swine heart preparation under conditions of global ischemia, no discernible benefits were achieved in hearts perfused with GIK enriched medium (32.4 mM glucose 0.025 units/ml insulin, and 5.9 mM potassium). Mechanical performance and glucose utilization deteriorated just as rapidly in treated as in untreated hearts (43). In addition, there was no improvement in survival time or tissue levels of high energy phosphates.

Since ischemic cells were unable to profit metabolically from extracellular carbohydrate enrichment other attempts were made to improve energy production by increasing the glycolytic flux intracellularly at glyceraldehyde 3-P dehydrogenase. Such an acceleration is theoretically possible by improving the cytosolic NAD/NADH ratio buffering excess hydrogen ion, and providing additional substrate to fuel the citric acid cycle. To test this hypothesis pyruvate and TRIS buffer were added to the coronary perfusate in separate studies using the ischemic swine heart model. In a group of 10 hearts 40 mM pyruvate buffered with TRIS was administered slowly into the coronary reperfusion loop over 15 minutes beginning after 15 minutes of control perfusion and just prior to the onset of ischemia. This allowed for complete mixing of these agents during the time coronary flow was being reduced and before the beginning of the ischemic study period at 30 minutes of perfusion. Another group of five hearts received only buffered TRIS (23 mM) and both treatment groups were compared with an untreated third group of nine hearts. Extra glucose fatty acid, and insulin were provided to prevent exhaustion of available substrates from the recirculating coronary perfusate over the course of perfusion. Immediately with the onset of electrical death or at the conclusion of 60 minutes of perfusion, portions of each heart were quickly frozen for later analyses.

Hemodynamic data from these hearts are shown in Fig. 1. Total coronary blood flow was reduced by a comparable amount in each group following 20 minutes of control perfusion. Clear evidence of ischemic changes in mechanical performance was observed in the untreated and TRIS treated groups as reflected by the marked rises in left ventricular end-diastolic pressures. In the pyruvate plus TRIS treated group however there was much less compromise in ventricular function and group survival time was significantly prolonged.

Metabolic comparisons are shown in Fig. 2. Oxygen consumption was terminally higher in the pyruvate plus TRIS treated group and somewhat lower in the TRIS only treated hearts. Glucose uptake was depressed to about the same extent in each group. Fatty acid oxidation, however was significantly lower with pyruvate suggesting that this substrate may preferentially feed into the citric acid cycle. Evans (44) initially described the influence of pyruvate on fatty acid oxidation in the aerobically perfused rat heart and reported both a decrease in

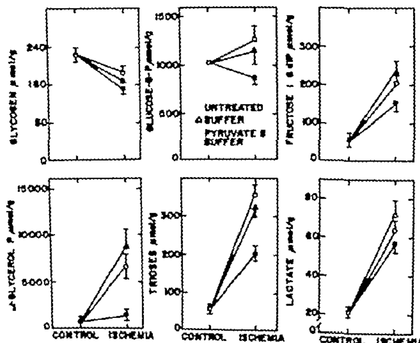


Fig. 3 Tissue levels of glycolytic intermediates in the same three heart groups. Control data in swine hearts perfused for 60 minutes at physiological flow rates are shown for comparison (□ symbol). Times of sampling correspond to group survival times as listed in Table 3. Ischemia resulted in declines in tissue glycogen and elevations in key glycolytic intermediates including fructose 1 6-P trioses, lactate and α-glycerol-P. In the group treated with pyruvate and TRIS glycogen utilization was increased and build-up of glycolytic intermediates was less, suggesting an increase in glycolytic flux rates during myocardial ischemia.

Table 3 Adenosine products in treated and untreated ischemic hearts.

Condition	Survival Time min	Creatine P µmol/g	ATP µmol/g	ADP µmol/g	AMP µmol/g
Control	60.0	48.3 ±3.1	1.4 ±.6	2.32 ±.08	.133 ±.020
Ischemia, untreated	24.3 ±3.1	24.2 ±2.4	16.9 ±.6	3.26 ±.18	.275 ±.029
Ischemia, treated with pyruvat and TRIS	36.8 ±2.5	25.7 ±3.0	16.9 ±.8	3.86 ±.30	.340 ±.060
Ischemia, treated with TRIS	30.0 ±5.1	27.3 ±1.8	16.0 ±.8	3.03 ±.14	.370 ±.077

Abbreviations as before. Survival times in ischemic groups were adjusted for the initial 20-minute periods of control flow. Control perfusions were terminated after 60 minutes. Coronary perfusate concentrations of insulin (.025 mUnits/ml), glucose (23.4 mM) and free fatty acids (.7 mM) were comparable between groups.

oxidation of palmitate-C[14] and increased incorporation of palmitate into tissue lipid, specifically in tissue triglyceride. This was accompanied by a two-fold acceleration in pyruvate decarboxylation. Although definitive proof of this reaction was beyond the scope of the present study, the same trends may also be occurring in ischemic myocardium, suggesting that pyruvate may serve as a preferred substrate for the residual oxidative metabolism that occurs in ischemic tissue. Although utilization of exogenous glucose was not significantly improved, addition of pyruvate plus TRIS did appear to alter glycolytic inhibition (Fig. 3). As compared with a group of six hearts perfused at control levels for 60 minutes, ischemia produced appreciable decreases in tissue glycogen stores and elevations in glycolytic intermediates including fructose 1,6-P, trioses (glyceraldehyde-3-P and dihydroxyacetone), lactate and α-glycerol-P. However, glycogenolysis was greater in the pyruvate treated group (lower tissue glycogen) and glycolytic intermediates were distinctly lower than in other ischemic groups, suggesting that glycolytic inhibition was partially removed. Despite a presumed increase in the conversion of pyruvate to lactate, tissue levels of lactate, although increased from control data, were not very different from other ischemic groups.

The oxidation of pyruvate and accelerated glycolytic metabolism in the hearts administered pyruvate and TRIS collectively served to preserve tissue energy stores as shown in Table 3. Ischemia reduced creatine phosphate and ATP in all groups as compared with control data but these changes were no greater in the pyruvate/TRIS group even though these hearts were exposed to global ischemia for a longer period (12.5 minutes longer than the untreated ischemic group).

Thus it seems possible that potential benefits may derive from metabolic manipulations of substrate consumption and utilization in ischemic hearts. Although more work is needed, both in this and other model systems, these preliminary data suggest that proper metabolic adjustments may help to salvage ischemic myocardium, prolong survival times, and improve mechanical and metabolic functions.

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DISCUSSION

Dr Hjalmarson

Thank you Dr Liedtke. I think this model is a complement to what we have seen before and it seems to be very useful.

Dr Mueller

In your study the lactate content of the ischemic myocardium was lower in the group receiving pyruvate and TRIS buffer compared to the group receiving glucose Do you think the addition of TRIS buffer decreased lactate accumulation in the ischemic myocardium?

Dr Liedtke

I was surprised that there was not more of a separation in the tissue lactate contents particularly since I expected in the treated group excess pyruvate to be anaerobically converted to lactate Part of the answer may resolve around the fact that in this sort of preparation as in all ischemia, oxygen availability is not an all or none phenomenon There is significant residual oxidative metabolism going on and it is just a question of what substrates are being used to accomplish energy production. The pyruvate clearly is undergoing oxidate metabolism in the citric acid cycle in these studies so much so that it was distorting the oxidation one would expect from fatty acid metabolism. TRIS even though not effective alone may also be contributing to pyruvate's effects in directing it preferentially into residual oxidative pathways

USE OF BABOONS IN STUDIES OF ACUTE MYOCARDIAL INFARCTION AND EFFECTS OF INFUSIONS OF GLUCOSE, INSULIN AND POTASSIUM (GIK)

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Ever since Hippocrates dissected apes to learn the secrets of primate anatomy it has been thought that man has a unique biologic relationship with the subhuman primates Galen wrote that of all living beings the ape was closest to man in the viscera, muscles arteries veins and nerves and in the form of the bones (1) We have begun to evaluate the anatomical electrophysiological and metabolic suitability of the baboon heart for studies of experimental myocardial infarction.

ANATOMICAL FEATURES

Hearts (weight 150-200 g) of adult mongrel dogs pigs and Cape Chacma baboons (*Papio Ursinus*) were injected via the aorta with unsaturated polyester resin containing a red pigment paste A complete cast of the coronary artery tree remained after treatment with concentrated HCl. In dogs there was usually a dominant and large left coronary artery with a small right coronary artery one third the diameter of the left coronary artery at the orifice There were many diagonal branches of the left anterior descending coronary artery and an extremely fine network of capillaries overlapping the supply regions of juxta-opposed artery branches (see Fig. 1)

In the baboons however we found a more balanced pattern of the supply between the right and left coronary arteries with the same diameter at the orifice and very few diagonal branches There was usually one major diagonal branch the third primary artery ( ) which took off either from the *proximal end of the left anterior descending coronary* artery or from the circumflex coronary artery There was no dense capillary network and no overlapping between the fine artery branches (see Fig 2) The patterns of the pig heart coronary arteries were between those of the dog and the baboon

Other differences between dog and baboon models are reported elsewhere (3) Infarcts in the baboons were produced by abrupt ligation of the distal third of the left anterior descending coronary artery aiming for an infarct approximately 10 % of the total heart weight. After ligation, there was a rapid darkening of the ischemic epicardial area with a sharp edge The distal part of the tied coronary artery remained empty there was no retrograde filling as usually seen in dog infarcts

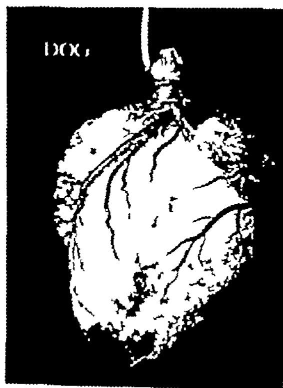


Fig. 1 Resin cast of the coronary artery tree of a healthy adult mongrel dog heart. The left anterior descending, the circumflex and several diagonal coronary branches are shown as well as the dense white capillary network.



Fig. 2. Resin cast of the coronary artery tree of a healthy adult baboon heart. The left anterior descending, the circumflex and the main diagonal or third primary branch are shown

ELECTROPHYSIOLOGICAL FEATURES

There was a high incidence of primary ventricular fibrillation (VF) in the first hour after coronary ligation (4). Of 38 baboons, 25 developed VF (66 %) within the first hour. Epicardial ST segment changes were recorded using a sweeping electrode and two different recording systems (5). One system consisted of a flexible stainless steel wire loop as exploring electrode and the Wilson central terminal as indifferent electrode using an AC amplifier. The other system consisted of 2 cotton wick pH calomel electrodes and a DC amplifier. Recordings were made from the basis of the left ventricle towards the apex and back parallel to the anterior descending coronary artery and from the lateral edge of the right ventricle towards the lateral edge of the left ventricle and back again about 5-6 cm parallel to the atrioventricular groove. Each sweeping epicardial ST-recording served as its own control recording in two directions along the same epicardial track. Sweeps were made before ligation and every 15 minutes thereafter (see Fig. 3 and 4). Both methods revealed 5 different and usually concentric epicardial electrophysiological areas.

- area 1 — centre of the infarct with maximal isopotential ST-elevation
- area 2 — peripheral infarct zone with centrifugally decreasing ST-elevation
- area 3 A — a narrow band 1 mm in width with an isoelectric ST-segment
- area 3 B — apparently normal non-cyanotic area surrounding the infarct with ST-depression and
- area 4 — distal apparently normal area with isoelectric ST-segment

The ST-elevation found in epicardial sites in areas 1 and 2 A was due to a simultaneous depression of the baseline and a true elevation of the

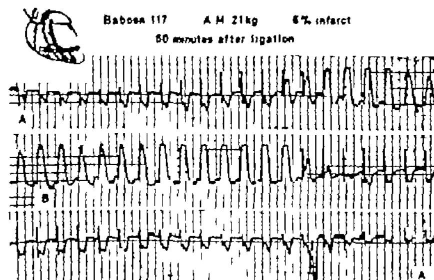


Fig. 3. Continuous ECG recording with the wire electrode and A.C. amplifier showing the 5 different electrophysiological areas recorded at a speed of 25 mm/sec and 1 mm = 1 mV

ST-segment. Total epicardial ST-segment was not stable during the 1st hour after coronary artery ligation and maximum ST-elevation was found at 60 minutes (no later recordings made) as recently reported for the pig (6). There was no correlation between the absolute height of ST-elevation in mV and the electrical severity of the infarct as judged by arrhythmias and the incidence of primary VF

METABOLIC STUDIES

By the use of labeled microspheres we found that the flow in the centre of the infarct (area 1) was 5-10 % of the preligation flow in area 2 flow was 20-30 % and in area 3 flow was about 70 % in non-fibrillating baboons and normal in fibrillating baboons (7). In 26 baboons the effects of glucose, insulin and potassium (GIK) infusions were studied (8). The infusion was started at 3 minutes after coronary artery ligation at a rate of 0.2 ml/kg/min. Twelve baboons received 100 g/L glucose with 40 *mEq KCl and 40 U insulin* 14 other baboons received 200 or 500 g/L glucose and 60 mEq KCl and 60 U insulin these two rates of provision of GIK gave similar results which were combined. The insulin was NUSO (Wellcome) and glucagon low Biopsies were taken from the heart 60 minutes after the coronary artery ligation and compared with 12 baboons who had a coronary ligation and an infusion of 0 45 g/L NaCl or no infusion The significant tissue biochemical changes due to GIK infusion are summarized in Table 1

Besides tissue metabolic changes the GIK infusion reduced circulating FFA by about half and increased diuresis The only significant effect of

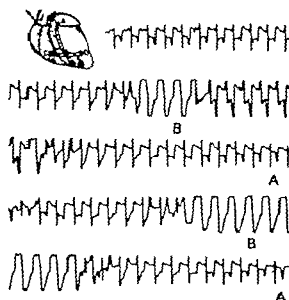


Fig. 4 Continuous ECG recording with the wire electrode and A C amplifier showing the 5 different electrophysiological areas recorded at speed of 25 mm/sec and 1 mm = 1 mV

GIK infusion on epicardial ST-segment changes consisted of prevention of the full development of ST-depression in area 3

We conclude that GIK had a beneficial effect and counteracted early tissue metabolic deterioration in the infarcting baboon heart However our results do not provide a simple answer about the active principle in GIK nor about the complex and probably multilevel effect of glucose insulin and potassium on acute myocardial infarction (9).

Table 1 Effect of infusions of GIK on tissue metabolic changes in baboon heart infarcts within 1 hour

Electro-physiological area	1 Infarct centre	2 Infarct periphery	3 B Peri-infarct zone	4 Distal zone
Glycogen	↑	↑↑	↑	↑
Creatine phosphate	↑↑	↑	=	=
K /N ratio	=	↑	↑	↑
ATP	↑↑	=	=	=
Homogenate pH	=	=	=	=
Inorganic phosphate	=	↓	=	=
Lactate	=	=	↑	↑

unchanged compared with non-treated ligation baboons

↓ increased or decreased compared with non-treated ligation baboons

increased early in relation to KCl-infused controls

For detailed results see Opie et al (8). The above results supplant preliminary results reported in 1972 (10).

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DISCUSSION

D Hjalmarson

Thank you very much Dr Bruyneel We have now time to discuss the papers with the swine heart and the baboon and I would like Dr Morgan to take the chair I think it might be of interest to discuss especially the value of glucose-insulin-potassium.

Dr Morgan

Do we have questions on either one of these papers I have to apologize for having heard only the last five minutes of the last one due to my late arrival so I am not in the best position to help in the discussion myself

Dr Mjos

Dr Bruyneel is the dose of glucose-insulin-potassium that you used the same as the dose the Braunwald group used in their studies with glucose-insulin-potassium?

Dr Bruyneel

I think the lower dose was 10 per cent glucose with 40 units of insulin and 40 meq of potassium chlorate I thought it was the same but I am not sure We had two concentrations The other was 50 per cent of glucose and 60 meq of potassium chlorate and 60 units of insulin which is the highest dose Sodi-Pallares is mentioning. We did not find any difference between the 12 baboons with low dose and the 14 with high dose but there was an effect of the glucose-insulin-potassium.

Dr Maroko

Why do you define the zone of ST-segment depression as an ischemic zone since your microsphere measurements showed that there was no reduction in myocardial flow i e no ischemia

Dr Bruyneel

It all depends what concerns the area with ST depression. We found it even hyperemic But it was hyperemic in only those baboons who fibrillated so we were thinking that it was due to large gradients across area two which has increasing ST-elevation and area three with steep ST-depression. It is a

question of semantics to say whether it is ischemic or not. In hospitals and clinics it is normally correlated with ischemia. Now here on these microsphere studies we should not call it ischemia. In the survival ones there was normal flow and in the ventricle fibrillated baboons it was hyperemic there was increased flow compared with the control values so maybe I should not mention it as ischemic

Dr Maroko

Is it possible that this area is completely normal – normal from the point of view that it is not ischemic – and that the electrocardiographic changes are in one way or another a reflection of electrocardiographic changes in the truly ischemic zone and consequently it could be considered as shown also by your metabolic studies an essentially normal zone In the case of the baboons that showed hyperemia they later fibrillated Could the reason be that this zone was stealing from the ischemic zone and that the hyperemia was the sign that ischemia in the centre of infarct was worse

Dr Bruyneel

That could be but we cannot say that area three was completely normal. Biochemically there were changes There was increase of lactate and inorganic phosphate There was a glycogen depression We can say in comparison with area four which was much further away which is on the base of left ventricle that there were clear differences So I do not think you can consider it as a normal metabolic area. There are signs of ischemia, metabolically too so there is a difference between area three and four on metabolism and epicardial ST segment

Dr Williamson

It seems to me that the microsphere method of measuring flow does not give the best definition of ischemia. Judging from your data, area three was indisputably ischemic There was an increase in lactate and a decrease in pH This seems to be the best definition of ischemia.

Dr Bruyneel

Do you mean pH in area three or in which area with ST-change?

Dr Williamson

Maybe I am misremembering the slide but was the pH not a little low even in area three?

Dr Bruyneel

Mildly decreased. But much less than in comparison with area one and two with ST-elevation There is a big change So we still consider area three as ischemic

Dr Morgan

Was the lactate content elevated in area three

Dr Bruyneel

Yes. It was mildly increased in the control ligated baboons

Dr Morgan

I wonder what significance of higher levels of tissue glycogen amounts to for outcome of the infarct in the baboons given the glucose-insulin-potassium. Do you think this higher level of glycogen is of significance

Dr Bruyneel

We know that when glycogen and sodium potassium ratio is low we are pretty sure to have tissue necrosis later on. So if we can prevent low levels of glycogen and sodium potassium ratio we may prevent severe tissue necrosis in area two but without a change in the centre of infarction I think we have to consider this area as a lost area. You only affect area two which has a decreasing ST-elevation area. So we think – but we have not done any histological examinations yet – that there was less tissue necrosis 24 hours later

CELLULAR AND MOLECULAR MECHANISMS INVOLVED IN CARDIAC CELL FUNCTION EFFECTS OF AN ANTIBIOTIC IONOPHORE

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INTRODUCTION

Cardiovascular diseases represent the major cause of mortality in the world. For example last year in the United States there were approximately 700,000 deaths due to myocardial infarction

During the past 10 years my colleagues and I have been concentrating our efforts on the cardiac muscle cell in order to understand the mechanism of control of excitation-contraction and relaxation coupling. W ha e stressed the various membrane sinks" that may modulate intracellular calcium. We have isolated, purified and partially characterized the Na K -ATPase associated with the cell membrane the sarcoplasmic reticulum mitochondria and contractile elements. A hypothesis for the mechanism of action of digitalis glycosides ha been developed. In addition we have been employing a new ionophoric antibiotic (a chemical agent that interacts with membranes and transports" ions into the cell) RO 2985 (X537A) which has a unique action on the cardiovascular system and may represent a new class of pharmacological agents that may have significance in the treatment of various disease states such as hemorrhagic and cardiogenic shock

A GENERAL DESCRIPTION OF THE CARDIAC MUSCLE CELL

Most investigators agree that the central cation in the control of the coupling of excitation to contraction and relaxation is calcium. With each depolarization a small but significant amount of calcium enters the cell presumably associated with the phase 2 of the action potential (Fig. 1). The transcellular flux of calcium with each beat may represent as much as 0 14 mm/kg of heart muscle/ minut It is interesting that approximately 25-40 μm of calcium/kg of wet weight muscle is required for full contraction (1,2). One of the kinetic phases appears to be associated with the superficial sites undoubtedly part of the cell membrane. In 1883 Ringer clearly demonstrated the requirement for an external source of calcium in order for the heart to function It is now well recognized that in contrast to skeletal muscle cardiac muscle needs both an external and an internal source of calcium. The latest concept of calcium control suggests that the cell membrane is the key controlling site and that sodium ions modulat both the influx and efflux of calcium. Using voltage clamp techniques Reuter

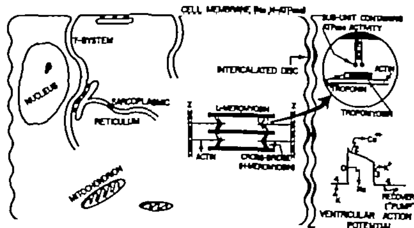


Fig. 1 Diagramatic representation of cardiac muscle cell. Insets, proteins involved in repression and derepression of crossbridge formation (top) and ventricular action potential (bottom).

has postulated that there exists in some membranes a carrier possessing two negative charges that can be occupied competitively by either two sodium ions or one calcium ion (3) The unloaded carrier does not move across the membrane or if it does it moves very slowly while the loaded carrier moves across very rapidly The loading of the carriers depends on the respective concentrations of sodium and calcium on either side of the membrane as clearly indicated in Fig. 2. Notice that the sodium gradient across the membranes controls calcium and this gradient is maintained by the sodium pump The sodium-calcium exchange system is electro-neutral, and the sodium gradient represents the only energy source for calcium extrusion and possibly influx This sodium-calcium exchange may represent a mechanism by which cardiac glycosides exert their action. The major effect of cardiac glycosides seems to be an inhibition of the Na k ATPase located in the cell membrane This causes the cell to gain a small amount of sodium

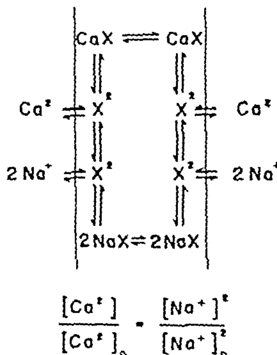


Fig. -- Carrier scheme for Na-Ca exchange acros membrane two N ion and one C ion compete for carrier (X') at the inside and outside surfaces of the membrane The carrier can move as 2NaX or as CaX across the membrane but the unloaded carrier cannot move. I such a transport scheme Ca gradient can be established across the membrane as consequence of existing N gradient The expecte distribution ratios at equilibrium are given by the equation below the scheme (Reproduced by perm sion of the American Heart A sociation, Inc (3)).

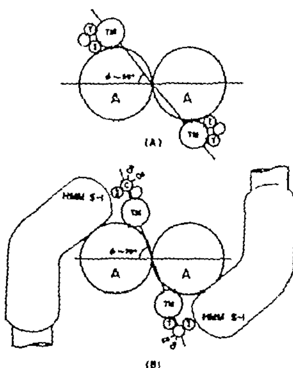


Fig. 3 Scheme of regulation of muscle contraction by TN and Ca2 The elative positions of actin, tropomyosin and the head of the myosin molecule (HMM S-1) in the model are essentially as proposed by Spudich *et al* (1972), Haselgrove (1972) Huxley (1972) and Parry and Squire (1973). Key A actin TM tropomyosin T Tn-T I TN-I C TN-C A elaxation in the absence of Ca^{2+} B activation $[Ca^{2}] \sim 1\mu M$ Suggested interactions between proteins are indicated by a short connecting line

if the internal sodium is increased for example from 10 mM to 15 mM it would cause more than a two-fold increase in intracellular Ca concentration Langer and his colleagues demonstrated that there appears to be a sodium-calcium exchange system which enhances calcium influx causing an inotropic effect in response to an increase in intracellular sodium concentration (4) Using an isolated guinea pig atrial preparation and a kinetic analysis of calcium Carrier *et al* showed that the fast exchanging calcium compartment is superficially located and is responsible for rapid changes in contractile force (5) Whether the cell membrane of the sarcoplasmic eticulum is the primary site for calcium control it is certainly clear that the modulation of heart function must occur through an exquisite control system moving calcium from contraction the troponin molecule All of this must occur in about 1/5 of a econd. The studies carried out in my laboratory during the past 15 years have dwelled on the enzymatic mechanisms for this control

It now established that the molecular site for

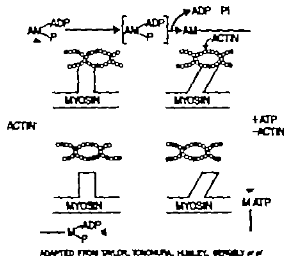


Fig. 4 Adapted from Taylor Tonomura, Huxley Gergely *et al*

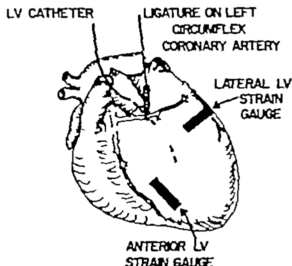


Fig 5 Model of occlusion-induced ischemia in dog heart.

contraction, concerned with calcium resides in the troponin-tropomyosin complex Calcium interacts with a specific site on the troponin molecule producing a micro-architectural change resulting in a de-inhibition of contraction, as pictured in Fig. 3 (7) Relaxation represents a process by which the sarcoplasmic reticulum becomes activated and sequesters (withdraws) all of the calcium from troponin. The energy for the contraction and the relaxation process (the latter also involves a calcium-ATPase) is derived from reactions carried out in *mitochondria*. The ATP is believed to react with the cross-bridge associated with myosin in the following manner (Fig. 4). ATP "breaks" the union between actin and myosin, after which a myosin-product complex forms $[M^{ADP}_{P}]$ actin then "activates" the complex and a series of conformational changes occurs which results in contraction and release of products [ADP+Pi] and the cycle begins again

CELLULAR AND SUBCELLULAR CHARACTERISTICS OF MYOCARDIAL ISCHEMIA

In order to study this complicated disease process, an experimental model is necessary so that samples of diseased and "normal" tissue can be taken from the same heart. The left circumflex coronary artery of conditioned dogs is ligated and contraction changes are recorded via strain gauges that are sewn on the epicardium of the lateral and anterior portion of the left ventricle Occlusion produces relatively discrete ischemia and subsequently an infarcted area in the posterolateral portion of the left ventricle particularly in the posterior papillary muscle while the anterior papillary muscle remains relatively normal (Fig. 5). Occlusion produces contraction changes and ECG changes that are characteristic (Fig. 6 7). We have emphasized ultrastructural aspects as depicted below Fig. 8 is a sample of the left anterior papillary muscle of a dog that was killed eight days after ligation of the coronary ar

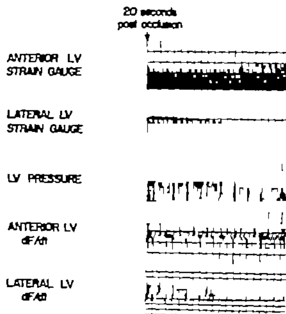
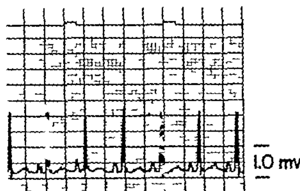
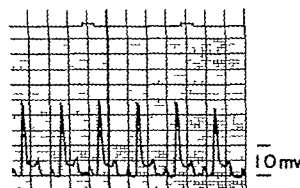


Fig. 6. Recordings of physiologic measurements, before and after occlusion of the left circumflex coronary artery Time scale each large division = 20 seconds, paper speed = 0.25 mm/sec.





3 MIN. POST OCCLUSION

Fig 7 Electrocardiogram (lead 11) before and after acute occlusion of the left circumflex artery

tery Note the normal arrangement of thick and thin myofilaments that formed the characteristic banding patterns. The distribution and size of the myocardium sarcoplasmic reticulum T system and glycogen are quite normal Fig 9 shows the control specimen for another dog Note A a region of contact at the intercalated disc between the cells of the left anterior papillary muscle which shows normal desmosomes and gap junction Panel B shows an intercalated disc of the left posterior papillary which is of course diseased The desmosomes in this ischemic tissue are significantly widened Fig. 10 show a perfused left posterior papillary muscle taken from a dog eight days after artery ligation Note that the Z discs are significantly widened and there are changes in the den ty Panel A show the return of an N line on one side f the Z disc but sometimes they appear on both ides Panel B shows the widened Z disc well s the N line on either side of the Z Note th dense material sembling Z substance that appears beneath the sarcolemma. An enlargement of Panel B is shown in Fig. 11 Other ultrastructural changes include swollen mitochondria containing dense deposits that have been described by Jennings fragmented filaments, distorted I bands and irregular Z bands with dense materials spread out over the thin filaments. Recently we have studied very early changes occurring after the ischemic insult. Jennings has indicated that in this particular model irreversible damage occurs after 18 to 20 minutes of ischemia. He has defined a series of sequential changes that are associated with mitochondria (Fig. 12) We have studied two time intervals 5 minutes and 10 minutes In general no signs of swelling, myofibril obstruction or nuclear clearing were observed in ischemic cells at these early time intervals Occasionally we observed profiles of fusing and budding mitochondria, large intramitochondrial granules having a ring-like appearance giant mitochondria, dense plaque-like material in the mitochondrial matrix and closely stacked cristae having either an angular configuration and arranged in a zig zag fashion across the mitochondria or mitochondrial flat profiles of cristae arranged in concentric whirls or U-shaped profiles (Fig. 13)



Fig 8 Portions of 2 myocardial cells in left anterior papillary muscle of dog killed 8 days after ligation of left circumflex coronary artery Normal arrangement of thick and thin myofilaments to form characteristic banding patterns normal size and distribution of mitochondria, sar oplasmic reticulum (SR) T ystem and glycogen are seen after perfusion fixation with glutaraldehyde (x 28 000 reduced by 33 %)



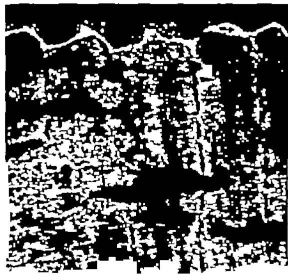


Fig. 9 Control specimen from another dog killed 8 days after coronary arterial ligation and perfused with glutaraldehyde fixative. A region of contact at intercalated disc between 2 cells of the left anterior papillary muscle shows normal desmosomes (large arrow) and gap junctions (small arrow). B intercalated disc of left posterior papillary muscle. The desmosomes in this ischemic tissue are widened. Compare with A at the same magnification (x 1_,000 reduced at 1_ %).





Fig. 10. Perfused left posterior papillary muscle taken from dog 8 days after coronary arterial ligation. Widening of Z discs and corresponding changes in density are shown. A shows appearance of 'N' line on one side of Z disc, but sometimes on both sides. B shows widened Z discs as well as 'N' lines on either side of Z. Dense material resembling Z substance is seen beneath the sarcolemma (arrow) (x 13,000 [A] and 10,000 [B] reduced by 11 %).



Fig. 11 Widened Z disc from same muscle shown in Fig. 10 at higher magnification. The 'N lines appear t have merged with the Z disc (x 40 000)

SARCOPLASMIC RETICULUM

We have developed isolation techniques and have extracted sarcoplasmic reticulum vesicles that are high in calcium-accumulating activity Using a rapid recording system we have shown that these membrane fragments are able to accumulate enough calcium and at a sufficiently fast rate to account for the process of relaxation in cardiac muscle (Fig 14). We have also found that protein kinase (cAMP and calcium-dependent) stimulates calcium uptake into sarcoplasmic reticulum

Sarcoplasmic reticulum was isolated from failing human and animal hearts and compared to normal human and animal hearts we found that the rate of accumulation and release phenomenon were both significantly depressed Accordingly we formulated a hypothesis concerning the possible biochemical cause of heart failure emphasizing a defect in the sarcoplasmic reticulum We have suggested in the past that alterations of intracellular pH may be responsible for the characteristics described

In acute cardiac ischemia, the very first change noted in the sarcoplasmic reticulum was a prolongation of the time to onset of calcium release We have noted that this sarcoplasmic reticulum preparation is not phosphorylated as well as control by protein kinase or phosphorylase b kinase

MITOCHONDRIA

Oxidative phosphorylation in mitochondria from ischemic tissue revealed a marked depression in all parameters measured The most impressive decrease was the reduction in oxygen consumption during State 3 respiration which indicates a severe

?
No Reflow
R flow
0 5 10 20 40 60 120 240
Occlude
Minutes

Fig. 1_ Modification of figure (11)





Fig 13 (A) I most of the cells the mitochondria vary more in size and shape than in unoperated dogs. Arrows indicate profiles of budding mitochondria. The matrix more condensed and the cristae form parallel arrays of angular membranes. Dense granules are found in the matrix. Lipofuscin granules resemble mitochondria. Experimental sample taken from left posterior papillary muscle after 4 minutes of ischemia (36,000)

(B) A portion of myocardial cell taken from the control left anterior papillary muscle from the same dog, which resembles the area shown in Fig 13 A. Note the large mitochondrion at the bottom (x 36,000)

impairment of electron transport capability. A difference spectroanalysis revealed significantly decreased absorption at 550 nm and 440 nm, compared to the level in normal. Addition of cytochrome c produced some improvement in the QO_2.

Carnitine stimulated oxidation of palmitic acid by mitochondria from normal and ischemic myocardium was determined. Oxidation of hexanoic acid by mitochondria was not affected by carnitine. Ischemia produced a significant depression of carnitine-stimulated fatty acid oxidation and the defect appeared to gradually diminish with time. We have recently examined oxidative phosphorylation in mitochondria isolated from heart muscle soon after coronary occlusion, namely 5 and 10 minutes. This time interval has not been previously studied. A significant depression of oxygen consumption supported by glutamate and succinate, as well as carni-

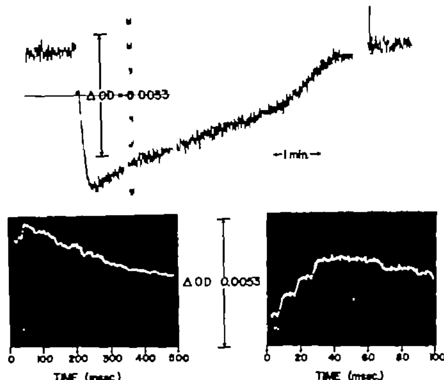


Fig. 14 Dual-beam spectrophotometric trace of calcium binding and release in cardiac relaxing system. Upper tracing shows entire cycle lower two tracings performed by stopped-flow show the rapid initial rate of calcium binding. Reaction medium: 0.1 M KCl 10 mM $MgCl_2$ 20 mM Tris maleate (pH 6.8), 0.2 mM Murexide 30 mM $CaCl_2$ 0.2 mM ATP at 30°C.

tine palmityltransferase I was noted as early as 5 to 7 minutes after the occlusion (Fig. 15 a & b).

Na K ATPase

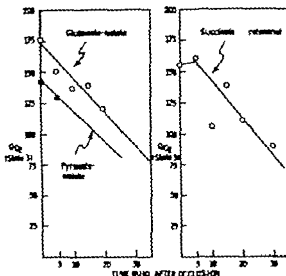
In this ischemic model we have not been able to detect a significant change from control until 7 days after ligation (Table I) We are repeating these studies, however using more purified enzyme preparations because it is well known that one of the first events to occur after ischemia is a loss of potassium from the cell and some investigators have reported depression of Na K -ATPase

CONTRACTILE AND REGULATORY PROTEINS

As early as 20 to 30 minutes after ligation, we have noted a defect in the calcium regulatory system and have localized this aberration in troponin

CONCLUSION

Although these studies are still preliminary and are continuing our present evidence strongly suggests that very soon after the ischemic insult significant changes occur in the various sinks for the control of intracellular calcium. We should direct our attention towards those intervention that might prevent or reverse these changes Accordingly we became interested in an antibiotic ionophore RO2 2985 (X537A) (Fig. 16) The substance has an interesting effect on all membrane systems, presumably exchanging calcium for a variety of monovalent ions However this agent can also transport or carry" all types of positively charged substances including catecholamines It has the interesting property of causing an immediate release of previously bound calcium to isolated sarcoplasmic reticulum. A single injection of this drug (RO 2985) into a catheter placed in the right atrium or into the femoral vein of an anesthetized animal caused a marked series of very interesting changes as described (Fig. 17 Table II) These changes particularly the prolonged increase in mean blood pressure and aortic cardiac output as well as the direct positive inotropic effect, are probably not due to a release of catecholamine We feel that a plausible explanation for the action of this drug is that it energizes a membrane-mediated calcium-proton or perhaps a calcium-sodium exchange that somehow leads to an increased availability of calcium. This includes vascular smooth muscle as well. Our most recent studies employ unanesthetized conscious animals that have been surgically fitted with indwelling chronic probes to measure regional blood flow contractile changes of the right and left ventricle cardiac output and most importantly coronary blood flow using an electromagnetic flow meter or Doppler



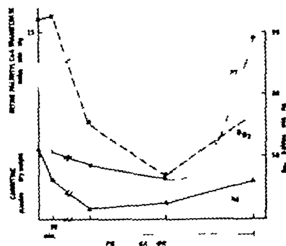


Fig. 15 (A) The effect of myocardial ischemia on respiratory function of dog heart mitochondria. Mitochondria were isolated from ischemic and control regions of dog heart at varying times following coronary occlusion. State 3 (ADP-stimulated) respiration was assessed using variety of mitochondrial substrates

(B) The effect of myocardial ischemia on tissue carnitine and carnitine palmityltransferase activity during early and prolonged periods of ischemia. Mitochondria were isolated from ischemic and control regions of dog heart. Mitochondrial carnitine palmityltransferase activity was measured and compared with mitochondrial respiratory function (glutamate-malate) at various time intervals following occlusion. Tissue carnitine levels were determined in homogenates from control and ischemic tissue samples

procedure. The results recorded after the injection of 1 mg/kg were quite striking: A modest increase in heart rate was noted and a very significant rise in systemic arterial pressure was recorded. This lasted

Table I. Na K -ATPase Activity Levels in Control and Ischemic Dog Heart*

Day	No	Control	Ischemic	
1	4	16.0	15.3	NS
7–8	8	17.5±2.9	10.4±3.9	P <0.01
>28	1	4.1	22.0	

Enzyme activity levels are expressed as μmoles of inorganic phosphate per milligram of protein per hour
NS = not significant, P <0.01 = highly significant.

for up to 7 to 9 hours. The heart was fitted with ultrasonic crystals which were placed in the wall of the myocardium approximately 1 mm from the endocardial surface to provide a continuous measurement (via the transit time principle) of the circumferential dimensions of a small (about 1 cm) segment of endocardial tissue. The crystal pairs were placed roughly parallel to the superficial fibers and the lead wires were tunneled subcutaneously and brought to the surface via a posterior cervical incision. These crystals were able to accurately record segment length changes. The drug produced a decrease in the systolic segment length with almost no change in diastolic length, and the decrease in systolic length was maintained for up to 3 to 5 hours. This means that there was a significant increase in contractility and indicates quite clearly that the drug produced a direct effect irrespective of the Starling mechanism. The results on regional blood flow and vascular resistance were quite interesting and of potential importance. Coronary blood flow increased prior to any other change and was independent of other effects. Both renal and iliac blood flows increased significantly. The time course of the changes is shown in Fig. 18. Several animals were placed in a severe hypotensive shock secondary to removal of about 1/3 the blood volume. After about 3 hours the systemic arterial blood pressure was down to about 55 mm of Hg. The administration of 1 mg/kg of RO 7985 produced an immediate six-fold increase in coronary blood flow followed by an increase in renal blood flow and then a steady but slow rise in blood pressure until 145 mm of Hg was reached. The usual type of increase in contractility was also noted with only a minimum change in heart rate. After a few hours, the blood was returned to

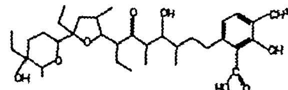


Fig. 16. Structure of RO? 2985

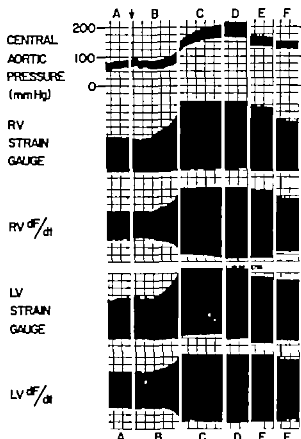


Fig. 17 Effect of RO2 2985 on hemodynamic parameters of an anesthetized dog The drug was injected via a catheter placed in the right atrium in a dose of 1 mg/kg at the arrow The record has been cut at various intervals. A DMSO control. B Time ero. C. 2 minutes. D 8 minutes. E. 20 minutes. F 75 minutes. RV = right ventricular LV = left ventricular and dF/dt = first derivative of force with respect t time Paper speed was 0.25 mm/sec.

two animals and both survived and are still living. This is the first example of an irreversible type of hypovolemic shock state that has been reversed completely This drug also shows some promise in the treatment of cardiogenic shock and possibly myocardial ischemia.

IS THE Na K -ATPase THE PHARMACOLOGICAL RECEPTOR FOR DIGITALIS?

Repke (12) was the first to suggest that Na K -ATPase may be a pharmacologic receptor for the digitalis glycosides. Work carried out by my colleagues and myself strongly suggests that no other organelle system or contractile proteins within the cardiac muscle cell functions as a receptor or binding site for cardiac glycosides Only the Na K ATPase actively binds the drug. To show this *in vivo* we carried out the following experiment A dog Langendorff preparation was administered with concentrations of ouabain to produce a minimum and maximum positive inotropic effect The hearts were removed and processed for Na K -ATPase sarcoplasmic reticulum and mitochondria. Only the Na K -ATPase was affected in a dose-responsive manner such that with a minimum positive inotropic effect there was a small but significant inhibition of the enzyme and with a maximum effect, but in the absence of toxicity the Na K -ATPase was inhibited to a greater extent (13) Recently however while a number of laboratories have reproduced these results Okita and his colleagues reported a dissociation of the positive inotropic action of digitalis from inhibition of Na K ATPase and suggest that the enzyme is an arrhythmogenic receptor" for digitalis (14). Unfortunately they used a rabbit preparation which binds digitalis in a relatively reversible manner while other species bind the drug rather tightly We repeated their experiments but included a highly sensitive species to digitalis namely the cat (15) At peak positive inotropic effect the

Table II Cardiovascular Effect of RO2 2985

	No. dogs	Control	Maximum values after RO2 2985	P
Mean blood pressure (mm Hg)	17	104± 7.4	148± 9.3	<0.01
Heart rate (beats/min)	16	150± 6 7	178± 7.8	<0.01
Aortic blood flow (liters/min)	11	1 9± 0.27	3.2± 0.37	<0.01
Vascular resistance (resistance unit)	11	59± 8.5	62± 9 7	NS
Left circumflex flow (ml/mi)	9	101±43 9	228±64 7	<0.01
Left ventricular force (%)	17	100	168±12.3	<0.01
Right ventricular force (%)	17	100	189±11.5	<0.01
Left ventricular dF/dt (%)	17	100	204±24.3	<0.01
Right ventricular dF/dt (%)	17	100	214±21 1	<0.01

All values are means ± SE P values were determined using paired Student t-test. NS = not significant.

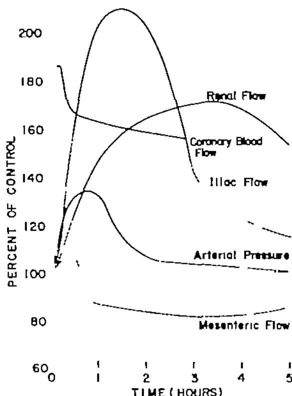


Fig. 18. Time course of RO2 2985 effects on systemic arterial pressure and regional blood flows. Values are average for all animals studied, calculated at 15 min intervals. Left circumflex coronary artery blood flow was studied in different group of dogs than were the other measurements shown and values were not obtained in this group after 3 hr Solid Lines = significant alterations from control values (p <.05 paired 't test) Dotted Lines = values not significantly different from control.

N K ATPase of the cat heart was inhibited, while at peak inotropic action the rabbit heart Na K -ATPase remained at control levels. This confusion was solved by using H³-ouabain, which indicated that at the peak inotropic action, most of the drug dissociated from the rabbit heart during isolation but the drug remained very tightly bound to the cat heart enzyme proving that the rabbit heart binds digitalis in a manner differently from the other preparations or at least the wash-off is much faster The rabbit is a poor species to use to attempt to correlate the pharmacological and biochemical ctions for the cardiac glycosides. These results suggest that the Na K -ATPase is indeed the pharmacologic receptor for digitalis How may the inhibition of the enzyme function? I the Introduction I discussed the concept of Reuter and Langer in which it is postulated that there exists a carrier in the cell membrane that can competitively combine calcium and sodium, and that the carrier moves across the membrane with great mobility when it is occupied by a ligand such as calcium or sodium but mo es across ery slowly or not at all when it is not occupied. If Na K -ATPase is inhibited small but significant increase of sodium occurs at the interside of the cell membrane This causes the carrier" to be occupied by greater amounts of the ligand and, hence a greater mobility of the carrier sodium complex which then migrates to the outside of the membrane liberating sodium and picking up calcium and releasing it on the inside of the membrane, thereby producing an increased force of contraction If the sodium is increased from approximately 10 to 15 mM then the increased amount of calcium that would reach troponin is estimated to be enough to produce a significant augmentation of contraction.

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This paper was submitted and accepted for publication but was not presented at the meeting due to author s travelling impediments.

CELL VOLUME REGULATION IN ACUTE MYOCARDIAL ISCHEMIC INJURY

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ABSTRACT

Thin freehand slices of left entricular papillary muscle of the dog, exhibit good cell volume regulation when incubated at 37° for 60 minutes in oxygenated Krebs Ringer phosphate solution. The fine structure of the cells is maintained throughout the incubation. This *in vitro* system was developed in order to test the capacity of myocardial cells irreversibly injured by 60 minutes of ischemia to maintain cell volume The results showed that irreversibly damaged cells were unable to maintain volume They swelled markedly lost Mg^{2} and K and exhibited structural defects in the plasma membrane of the sarcolemma. These observations establish that *loss of cell volume regulation s one of the early* events associated with the development of irreversibility in severe myocardial ischemic injury

Cell volume is maintained within narrow limits in healthy well differentiated cells through a complex mechanism involving active metabolism, variable ionic permeabilities osmotic and other factors (1 3 12 14). This system is sensitive to injury drug therapy and decreases in energy supply and usually responds by a reversible increase in cell water and sodium, and a decrease in cell potassium. Since acute ischemia is associated with a depressed net supply of ATP it seems likely that myocardial cell volume regulation would be altered *in vivo* in acutely ischemic cells However the expected changes in volume regulation have not been demonstrated early in ischemic injury in the intact animal.

Two technical problems have made *in vivo* study of sequential acute changes in volume regulation difficult in ischemia. First and most important is the fact that the variably depressed arterial flow of ischemia makes it virtually impossible to estimate the proportion of water confined to the intra- or extracellular compartment by the use of markers such as inulin Without knowledge of compartmental water distribution it is impossible to estimate cell volume *in vivo* (13). Volume measurements are confounded further by the probability that changes in cell membrane permeability occur in ischemia when and if present, alterations in permeability might further compromise estimates of intracellular fluid volume

Although acute changes in water distribution have been difficult to study *in vivo* it is known that lethal cell injury is associated with marked defects in cell volume regulation Necrotic myocardial cells have no volume control and exhibit an electrolyte distribution similar to that of the extracellular fluid (5 6 16) Levels of K and Mg^{2} are low while Na Cl and H_2O are high The dying cells eventually lose their identity and become a part of the extracellular space.

The experiments reported in this paper were made in an attempt to ascertain if changes in cell volume regulation occurred early in lethal myocardial ischemic injury in the dog heart. Estimates of the capacity of cells to control their volume were made *in vitro* on thin freehand slices of papillary muscle. The results have shown that loss of volume regulation occurs early in lethal acute myocardial ischemia, much earlier than it can be detected *in vivo* by direct tissue analysis (1 6, 16). Moreover our findings indicate that defects in volume regulation are one of the earliest changes observed to be associated with the shift from reversible to irreversible ischemic injury The pathogenesis of the defect in volume regulation remains to be established as does its relationship to the development of irreversibility

MATERIAL AND METHODS

Left ventricular myocardium contains much more K and Mg^{2} than are found in plasma while Na Cl^{-} and Ca^{2} are less concentrated (6 13). Myo-

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cardial water in health is strikingly constant comprising 78.6 to 78 8 % of the wet weight. Changes in cell volume are associated with alterations in both tissue water and weight therefore it is essential in cell volume studies to refer water and electrolytes not to the wet weight of the tissue but to a value assumed to be constant i e the tissue dry weight (3) The usual electrolyte and water content of the posterior papillary muscle (PP) of the left ventricle are shown in Table 1

Tissue Slices A tissue slice suspended in oxygenated neutral Krebs Ringer phosphate (KRP) solution, is comprised of a group of myocardial cells no longer dependent on a vascular system to provide O_2 and metabolites. Diffusion of oxygen into the tissue is limited only by its thickness (17) As long as the slices do not exceed 0.5 to 0 75 mm in thickness metabolism is not limited by O_2 diffusion, providing that the slices are mechanically agitated to facilitate O_2 diffusion, and as long as media O_2 saturation remains maximal

Slices cut parallel to the long axis of the papillary muscles function best probably because fewer cells are damaged when they are cut parallel to their long axis or perhaps because slices are easier to cut in this direction. Also our slices are cut freehand using sharp razor blades according to a technique described in detail elsewhere (3) Freehand cuts are made on a small block of papillary muscle which is held gently between the thumb and forefinger with the long axis of the cells parallel to the direction of the cut Crushing of the tissue must be avoided. In fact slices of left ventricle prepared with a Stadie Riggs tissue slicer do not function as well as freehand slices presumably because myocardial cells are damaged by crushing during the slicing procedure (3). By definition cells damaged by cutting are present on each of the six sides of the freehand slice Thus our measurements are being made on the uncrushed cells internal to the cut edges of the tissue Accordingly the limits of the slicing procedure are represented by the facts that very thin slices have a greater proportion of damaged cells and no diffusion defect while thicker slices have fewer damaged cells per unit volume but may exhibit defective O_2 diffusion into the cells in the middle of the slice

Water and electrolytes of slices and myocardium were estimated by standard techniques which have been described previously (3) Briefly water was measured by drying to constant weight at 105°C and electrolytes were measured by flame atomic absorption spectroscopy after extraction of electrolytes from the dried slice with 0.75 M HNO_3 (7 16). The inulin diffusable space was measured using ^{14}C labelled inulin (3) and is reported as the ml of water containing inulin per gram of dry slice

Electron Microscopy Portions of the middle region of the slices were fixed in glutaraldehyde and post fixed with buffered osmium. These were embedded in epon and sectioned with diamond knives according to procedures described previously (1 3 9). They were examined with a Phillips 200 or an Hitachi HU 12 electron microscope.

Myocardial Ischemic Injury Adult healthy mongrel dogs which had been starved overnight were

Table 1 Water and Electrolytes of Left Ventricular Myocardium

	Water	Na	K	Mg[2]	Inulin Space
	ml/g dry weight	mmol/100 g dry weight			ml/g dry weight
Control papillary muscle (5)	3.67 ±0 05	20 5 ±0.48	38.3 ±1.0	4.8 ±0.7	1 1
Freehand slices of papillary muscle (37°C 60 min) (6)	3 7? ±0 11	61 9 ±7 4	?9 9 ±2.0	4.. ±0.2	1 41 ±0 11
Freehand slices of papillary muscle (0°C 60 min) (3)	5 ?9 ±0 18	81 9 ±14 8	7 6 ±1	4.3 ±0..	

The number of dogs is given in parenthesis Part of the data in this Table was abstracted from reference 1 with permission of the Editor of J Mol C B Cardiol

Based on fat-free wet weight analysis from five anesthetized control dogs (AMA Arch Pathol 63 586-592, 1957 64 10-16 1957) the Mg data is not corrected for fat and comes from Am. J Pathol. 67 417-440, 1972.

The inulin space was not measured in these dogs but is based on the sodium space A ssuming that the sodium space data is roughly equivalent to the inulin pace in heart the inulin space would be about 1 1 ml/g dry tissue N ie no inulin space data in the papillary muscle of the dog is known to the author

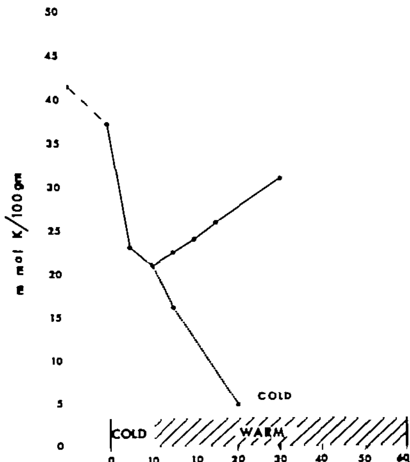


Fig. 1 Effect of 10 minutes cold incubation on K of papillary muscle slices. Freehand slices were incubated at 0-1°C for 10 minutes. Water N K and Mg^{2} were measured in triplicate at each point. The depressed metabolism produced by 10 minutes of cold was associated with decrease in slice K of 16 mmol/100 g dry tissue. Continued cold incubation decreased tissue K to 5.5 mmol K/100 g dry weight. Resumption of metabolism after 10 minutes (solid line) raised K from 21 6 to 31.5 mmol, gain of 9.9 mmol or ~60 % of the K lost. Prolonged periods of incubation did not cause greater increase in K The simultaneous changes in Na^+ and H_2O are show in Fig. Mg^{2} concentration remained constant throughout the incubation. (Dog 2123). Unreported data from RBJ and M L. Hill.

anesthetized with intravenous sodium pentobarbitol. Respiration was maintained with a Harvard model 1063 respiratory pump at a mean rate of about 300 ml room air per minute per kg body weight After opening the left chest the circumflex artery was ligated immediately distal to the first atrial branch at a distance of 5 to 15 mm from the origin (9 10). Six dogs were occluded for sixty minutes. At the end of the desired time interval, the heart was excised and was placed in about 750 ml of ice cold isotonic KCl surrounded by crushed ice In control dogs the heart was excised quickly without occlusion. After two – three minutes of cooling, blocks of left ventricle containing the PP and anterior papillary muscle (AP) were excised from the heart and were transferred to smaller beakers filled with ice cold KRP Freehand slices were prepared from the damaged PP and from nonischemic control tissue (AP). The methods of infarct production have been discussed in detail elsewhere (9 10). However only gray tissue of the type illustrated in Fig. 3 and Ref 10 was used for PP slices. Furthermore any gray irreversibly damaged tissue found in the AP was excised prior to preparing slices.

RESULTS

Cell Volume Regulation *In vitro*
Effect of Metabolic Inhibition in Control Tissue

Myocardial tissue slices incubated at 37°C for as long as three hours maintain a water volume of 3 72 ± 0 11 ml H_2O/gram of dry tissue. This volume is equivalent to that measured in whole tissue of the same animal (Table 1) The inulin diffusable space comprises about 1 4 ml or about 38 % of the total tissue water a value about 8 % greater than that estimated by the chloride or sodium space *in vivo* (6, 13). Inulin equilibrates with slice water in about 20 minutes.

Na K and Mg^{2} content of myocardium are given in Table 1 The Mg^{2} of freehand slices, after one hour at 37°C in KRP is virtually identical to the whole tissue On the other hand, K is roughly

two-thirds as great and Na is doubled to tripled. The increase in slice Na is believed to be due to the fact that the cut edges of the slice are bathed in fluid high in Na and because slicing itself produces an increased interstitial space secondary to a reduction in tissue tension. The lowered K is due partially to the presence of cut cells on the edge of the slice being in equilibrium with KRP solution with a low K concentration.

The effect of inhibition of metabolism by cold on slices of normal myocardium is shown in Fig. 1 Ten minutes at 0-1°C resulted in a loss of 16 mmol K from the slice much of which was regained over the next 30 minutes if the slices were rewarmed to 37°C. The loss of K is believed to be a passive phenomenon occurring because cold inhibition of metabolism provides no energy to pump Na from the cells. Other slices from the same animal (labelled cold) demonstrate the effect of prolonged inhibition of metabolism. Only 30 minutes were required for the K of the slice to reach its lowest point, about 5 mmol K/100 g dry tissue

The increase in Na and H_2O caused by cold inhibition of metabolism is shown in Fig. 2. The water increased by about 21 % in only 5 minutes in the cold while the Na increased by over 100 % Sodium and water increased even more if metabolic inhibition was maintained for 60 minutes. However rewarming the slices resulted in a return of water to control levels in about 30 minutes and in a simultaneous prompt decrease in slice sodium content. Grachowski *et al* (3) have shown that these changes occur without any alteration in the inulin space, a finding which suggests that H_2O shifts are occurring in a compartment of the slice which is free of inulin, a molecule with a molecular weight of 5000 This compartment is assumed to be the cellular compartment.

The fine structure of cells in the center of the slice remains intact throughout 60 minutes of incubation at 37°C (Fig. 3) These cells were virtually indistinguishable from those of control tissue of the same animal fixed by immersion prior to slicing. In particular glycogen was abundant nuclear chromatin was distributed evenly and mitochondria myofibrils and the sarcolemma appeared to be intact (9).

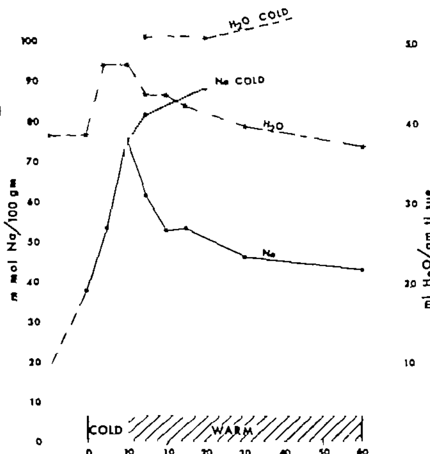


Fig 2. Effect of 10 minutes cold incubation of Na and H_2O of papillary muscle slices. Ten minutes in the cold caused total tissue water (TTW) to increase 29 % i e. from 3.8 ± 0.1 to 4 7 ± 0 1 ml/g After 30 minutes of metabolic activity (labelled H_2O) TTW had returned almost to the control levels and after 60 minutes of metabolism, the water did return to control. On the other hand, continued xposure to cold for 60 minutes increased tissue water by 50 % to 5 6 ± 0 1 ml N increased by 100 % after 10 minutes in the cold but this level reached 90.4 mmol/100 g or almost three times control levels after 60 minutes in the cold. Resumption of metabolism after 10 minutes in the cold (Na) caused prompt loss of Na t 47 4 ± 2.8 at 30 minutes and 44._ ± 5.2 mmol/100 g dry tissue at 60 minutes. The dotted lines between the ordinate and zero on the abscissa are drawn between the Na and H_2O content of the whole tissue and the first slices to be placed in media. (Dog 2123). Unreported data from RBJ and M L. Hill

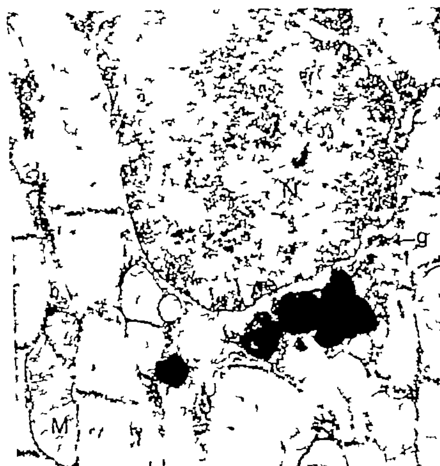


Fig. 3 Freehand slice of anterior papillary muscle (AP) incubated *in vitro* for 60 minutes at 37°C. Part of representative cell from the center of the slice is illustrated. The chromatin of the nucleus (N) is dispersed evenly as it is in control non-incubated tissue. Mitochondria (M) show minimal or absent swelling. Glycogen (g) is abundant. Lysosomes are intact. The Z bands are not thickened. Tissue incubated for 60 minutes at 0-1°C is indistinguishable from the figure illustrated here (Mag X 16,400). Reproduced with permission of the Editor of J Mol. Cell. Cardiol. (1).

Cells swollen in the cold showed no distinguishing features. The matrix space of the mitochondria appeared to be increased slightly but this change was not marked enough to be objectively detectable (1 3). Stereological techniques ha e not been applied

Cell Volume Regulation in Ischemic Tissue

The data in the preceding section of this paper establish that slices of control myocardium at 37°C in oxygenated KRP maintain a constant water content, and an inulin space comparable to that noted *in vivo* Moreover increases in slice water and Na and decreases in slice K associated with cold inhibition of metabolism are reversible to a significant extent by resumption of metabolism. In addition slice ultrastructure is maintained over long periods of incubation even though no exogenous substrate is added to the media. The capacity of tissue irre versibly injured by 60 minutes of ischemia to maintain cell volume in comparison to nonischemic AP of the same dog was tested in the experiments reported in the following paragraphs.

H_2O & Electrolytes Cells of the PP did not exhibit changes in water and electrolytes after 60 minutes of ischemic injury The levels of Na K Mg^{2} and H_2O reported under PP infarct, *in vivo* in Table 2 were virtually identical to those noted under control papillary muscle in Table 1 However electrolytes and H_2O of the PP are greatly altered if periods of ischemia are prolonged to 8-24 hours (6) Presumably no changes are noted *in vivo* after 60 minutes of permanent ischemia, either because electrolyte and H_2O content are maintained at control levels during much of this period, i.e. during the phase of reversible injury or because the low collateral flow in the PP slows diffusion of electrolytes and water and prevents detection of marked alterations in intracellular electrolyte distribution (10).

The data in Table 2 compares the electrolytes and water in slices of control muscle to those found in the PP infarct Note the marked increase in slice water the very low level of K and Mg^{2} and the high level of N in PP in comparison to slices of the AP Also the inulin diffusable space of the PP was increased significantly (Table 2) Note that the

Table 2. Cell Volume Regulation *In Vitro* in Tissue Slices of Control and Irreversibly Injured Myocardium[1]

	Water	Na	K	Mg[2]	Inulin Space
	ml/g dry weight	mmol/100 g dry weight			ml/g dry weight
Control (AP) slices 37°C 60 minutes	3.72 ±0.11	61.9 ±7.4	29.9 ±2.0	4.2 ±0.2	1.41 ±0.11
Ischemic (PP) slices 37°C 60 minutes	4.26 ±0.10	90.8 ** ±2.9	11.3 ** ±1.7	2.6 ±0.2	1.96 ** ±0.2
PP infarct *in vivo* at 60 minutes	3.46 ±0.40	19.8 ±1.7	38.8 ±1.3	4.3 ±1.[illegible]	

Data on AP and PP slices used with permission of the Editors of J. Mol. Cell. Cardiol. (reference 1). Myocardium of posterior papillary muscle (PP) was damaged by 60 minutes of ischemia *in vivo* prior to slicing. The probability that the *difference between AP and PP slices is statistically significant by a two-tailed paired t test is ** p <0.01 or ** p* <0.001

Based on analyses for H_2O, Na, and Mg^2 from 10 anesthetized control dogs (Am. J. Pathol. 67: 417-440, 1972). The K values are from reference 6 and were obtained from 4 dogs.

PP tissue (PP infarct at 60 minutes) showed no defects in H_2O or electrolytes when analyzed prior to slicing (Table results demonstrate clearly that irreversibly injured myocardium was unable to maintain its volume when it was placed in an infinite volume of KRP).

Fine Structure. Although cells irreversibly injured by 60 minutes of severe permanent ischemia showed no changes in electrolytes and H_2O, they did show marked changes in ultrastructure (9). These have been described in detail elsewhere and are shown in Fig. 4. The most striking changes are:



Fig. 4. PP after 60 minutes of ischemic injury *in vivo*. Parts of two cells and capillary are shown. The myofibrils are relaxed. I bands are prominent. Mitochondria (M) are enlarged due to an increase in matrix space. Amorphous matrix densities (a) are prominent *in most* mitochondrial profiles. At this magnification the sarcolemma appears intact. The capillary endothelium shows no abnormalities aside *from a decrease in* pinocytotic vesicles. Osmium fixation stained with uranyl acetate and lead citrate (Mag X 12,700). Reproduced with permission of the Editor of J. Mol. Cell. Cardiol. (1).

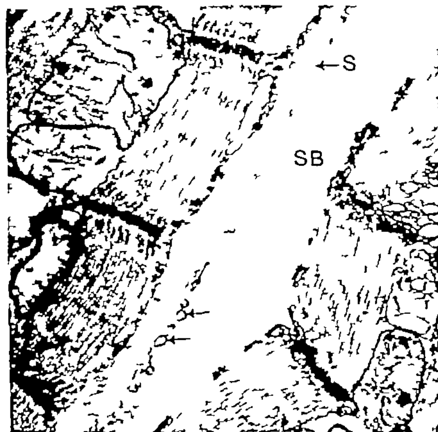


Fig. 5. Slice of PP shown in Fig. 4 after 60 minutes incubation at 0-1°C. TTW was increased by more than 50 %. The swelling included formation of a subsarcolemmal bleb (SB) which is of interest because the plasma membrane of the sarcolemma is incomplete over the bleb. The irregular circular profiles are believed to be remnants of the plasma membrane (arrows). The basement membrane of the sarcolemma appears intact (S). The myofibrils have remained relaxed. The mitochondria appear swollen and contain no densities aside from the amorphous matrix densities which were present prior to incubation (Mag X 25,200). Reproduced with permission of the Editor of J. Mol. Cell. Cardiol. (1).

mitochondrial swelling and occasional mitochondrial fragmentation appearance of amorphous mitochondrial matrix densities virtual absence of glycogen and margination of nuclear chromatin. When slices of damaged PP were incubated at 0-1°C in KRP for 60 minutes the cellular changes remained identical to those seen *in vivo* with the exception that the cells appeared to be swollen (Fig. 5). The swelling often was so marked that the sarcolemma was lifted off the myofibrils, leading to the formation of a subsarcolemmal bleb. Sarcolemma over the blebs often showed striking defects in the plasma membrane of the sarcolemma (Fig. 5) (1).

Incubation of PP slices at 37° for 60 minutes resulted in two additional findings not seen in tissue incubated in the cold (Fig. 6 & 7). The mitochondria of some cells showed granular densities of the calcium phosphate type Fig. 7. Furthermore these small cells usually showed contraction of sarcomeres with thickening of Z bands (Fig. 6).

DISCUSSION

The results of these experiments show clearly that the capacity of myocardial cells to maintain cell volume can be assessed by an *in vitro* technique. General limitations of the technique have been described in detail elsewhere (3, 17), and the limitations pertinent to studies of ischemic injury have been discussed earlier.

There appears to be little doubt that cell volume regulation is absent in most if not all cells which just have entered a state of irreversible injury. The nature of the defect remains unknown. It does not seem likely that it is due to absence of substrate because addition of succinate, a substrate readily metabolized by slices of ventricle (1, 15) failed to alter the defect in cell volume control. Since much of the intracellular Mg^{2+} is bound to adenine nucleotide, the low tissue Mg^{2+} in the PP suggests that tissue adenine nucleotide supplies may be depleted (1). Inadequate ATP supplies could interfere with the operation of the sodium pump. However, slice nucleotides have not been estimated in this model.

A striking and unexpected change in the swollen cells were the plasma membrane defects seen in Fig. 5. These may represent "holes" through which a molecule the size of inulin can penetrate the cell water. These defects are believed to have been present *in vivo* prior to placing the tissue in the media.

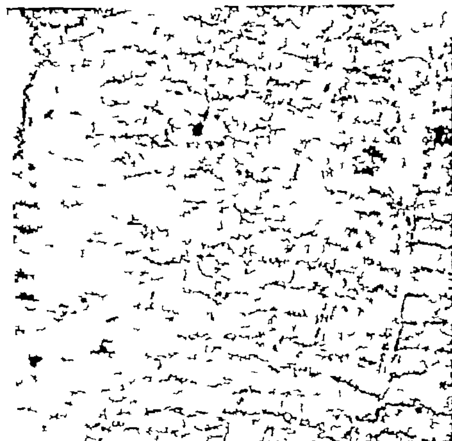


Fig. 6. Slice of damaged PP of Fig. 4 incubated for 60 minutes at 37°C Note the irregular contraction of myofibrils These structures are shown in the relaxed form exhibited *in vivo* in Fig. 4 I bands have disappeared, only thickened Z bands are present (Mag X 6000). Reproduced with permission of the Editor of J Mol Cell Cardiol (1)

If they were they have been difficult to detect unless the affected cells have been allowed to swell (9 11) At present, they have been seen in myocardial cells irreversibly injured by an episode of 40 minutes of ischemia and allowed only five minutes of arterial reperfusion (1) Such cells swell almost explosively (Fig. 8) and commonly exhibit a defect (Fig. 9) virtually identical to that seen *in vitro* (Fig. 5) A similar change has been described by Ganote *et al* in rat myocardium injured by 5 minutes of anoxia with continuous perfusion in a Langendorff apparatus (2) Reoxygenation of cells injured in this fashion is associated with the loss of much creatine phosphokinase (CPK) marked cell swelling, and



Fig. 7 Slice of damaged PP incubated for 60 minutes at 37°C Note that both granular (g) and amorphous matrix densities (a) are present The granular densities are believed to be calcium phosphate Note the irregular thickening of the Z bands (Mag X 47,000) Reproduced from Ref 1 with permission of the Editor of J Mol. Cell Cardiol



Fig. 8. Massively wollen cell of PP which was injured by 40 minutes of ischemia *in* *h* and then allowed tw minutes of reflow. Note the contraction bands (CB), large intracellular vesicles (V) and swollen mitochondria. A subsarcolemmal bleb is show at the bottom (S) (Mag X 6000). Reproduced from Ref. 11 with perm sion of the Editor of Am. J Pathol.

similar membrane defects (? 4) It seems likely that the defects m membrane permeability or defects of the plasma membrane of the sarcolemma itself may be ery early or perhaps the primary event causing the transition from reversible to irreversible mjury in ischemia (8)

The electron microscopic findings observed m the irreversibly injured cells incubated at 37°C provide objective evidence that the cells in the slices of irreversibly injured tissue are swollen and suggest that most if not all of the increase in slice water is due to cellular swelling. On the other hand, if one assumes that any tissue water penetrated by inulin is part of the extracellular space then the cell water estimated to be present in control and injured slices in Table _, i.e. the difference between total H_2O and inulin space has remained constant at about _30 ml/g dry tissue The probable explanation for our failure to detect an increase in cell water with inulin is the fact that the cells with large defects in the plasma membrane essentially have become a part of the extracellular space However these cells still contribute dry weight to the slice and thus effect ely reduce the total tissue water per gram even though many swollen cells still not penetrated by this large marker (inulin) are present. Thus, it seems likely that the large plasma membrane de fects lead to the increase in IDS observed in Table 2, while other injured cells in the slice simply are swollen, nonfunctional and free of inulin.



Fig 9 Sarcolemmal bleb in dog treated similarly to that illustrated in Fig. 8 but after 5 rather than minutes of arterial reperfusion. The usual architecture of the sarcolemma is shown at (S). Note that the plasma membrane of the sarcolemma is incomplete (arrow) and exhibits an appearance similar to that show in Fig. 5 (Mag X 1,000). Reproduced from Ref. 10 with permission of the Editor of Am. J Pathol.

It is clear that much remains to be learned about cell volume regulation in ischemia. At present, the *timing of the onset of the defect in cell volume* regulation found in the slices of irreversibly injured tissue is not known Moreover studies of the capacity of reversibly injured cells to maintain volume have not been made

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Saideh Safavi and Jonathan Worstall provided skilled technical assistance during the course of these experiments.

DISCUSSION

Dr Morgan

I think we might take some specific questions on Dr Jennings paper and then have a more general discussion about this and Dr Williamson s paper at the end of this portion of the program Are there any specific questions dealing with this presentation?

Dr Thomas

Does beta-blockade alter the tissue water values during ischemia?

Dr Jennings

I have never tested propranolol effects *in vitro* Since propranolol therapy does reduce the amount *of necrosis found in vivo it seems likely that less* severe defects in cell volume regulation would be observed *in vitro* in slices made from treated ischemic animals. *Until this system is studied in detail in vitro* we will not know its capacity to detect minimal defects in cell volume regulations.

Dr Braunwald

Do you think that the changes that you described occur as a consequence of irreversibility?

Dr Jennings

As you know Dr Braunwald, we have detected a number of different kinds of changes in this system as it passes from a stage of reversible to a stage of irreversible injury We are rather pleased with this particular change but we have not proved and we have not thought of a way of proving that this defect is a primary defect. I like it much better than the mitochondrial hypothesis I proposed several years ago

Dr Braunwald

You like it as which? As a cause of irreversibil ty or as reflection of irreversibility?

Dr Jenning

That I cannot simply answer We are dealing with a biologic system and attempting within this system to determine whether it is the cause or not. I am not certain that we can determine that One can only establish this after making a hypothesis study whether this is the cause and then by altering it see if you can prevent it.

Dr Maroko

Since there is no discernible difference in these parameters *in vivo* but only *in vitro* it could be hypothetized that this is just a potential defect that did not yet occur *in vivo* Therefore it is more likely to be a consequence than a cause of it.

Dr Jennings

I think that I have shown you that *in vivo* we can detect precisely the same set of changes by simply allowing a re flow so that the defect is a defect which occurs *in vivo* if you create the same experimental circumstances The only other way I can answer the question is to say that when one does this *i vivo* the affected cells, the irreversibly injured cells and cells with the nuclear and the mitochondrial changes swell become necrotic and die and will be replaced by scar tissue Beside that I have no way of defining biologically irreversibility I should in passing note that irreversibility is rarely defined in the current literature. This is a definition and you can either take or leave it.

Dr Morgan

I had one thing that was bothering me and that was the interpretation that the intracellular volume was increasing when you incubated slices *in vitro* When the slide was on I did a subtraction of the inulin space from the total water and found exactly the same value in the normal and the ischemic cell. Your conclusion is based therefore on the assumption that inulin is going into some of the ischemic cells but not into the normal cells

Dr Jennings

I think that is correct

Dr Morgan

I wonder if you tried to wash the inulin out.

Dr Jenni gs

No we have not tried to wash inulin out. Inulin is a difficult material to deal with within this particular system because one is dealing with the problem of diffusion of the inulin in the slice versus the rate at which the changes are developing.

Dr Morgan

What is the evidence that the inulin is actually inside of any cell

Dr Jennings

There is no evidence that inulin is inside any cell The best evidence that the cells are swollen is the electronic-microscopic evidence

D Morgan

We tried the same kind of experiment in perfused heart in the past and found that in anoxia and in cold perfusion virtually all of the swelling took place in the extracellular space at least as judged by sorbitol distribution. If the heart was reperfused the sorbi tol was washed out very rapidly suggesting that the extracellular volume was increased and not the intracellular volume

Dr Jennings

I think that if you tried to wash it out of these cells with the holes in the plasma membrane you would wash it out and you would reach the wrong conclusion

EFFECTS OF ACIDOSIS ON MYOCARDIAL CONTRACTILITY AND METABOLISM

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SUMMARY

The effects of increased H^+ concentration and the competition between H^+ and Ca^{2+} on cardiac contractile function and metabolism have been investigated using the perfused rat heart. A working heart preparation was established by cannulating the aorta and left atrium. Fluid ejection from the left ventricle passed into a small closed air space and escaped through the coronary circulation, thereby allowing a minimum dead space between changes of perfusion fluid. Respiratory acidosis (high pCO_2) to pH 6.6 produced a rapid fall of left ventricular pressure with a half time of 5 sec. This effect could be fully counteracted by an increase of the Ca^{2+} concentration in the perfusion fluid. Calcium titration curves against left ventricular pressure are shown illustrating a shift of the curves towards higher Ca^{2+} concentrations with decreased pH or verapamil addition and a shift towards low Ca^{2+} concentrations with epinephrine. In contrast to effects obtained with respiratory acidosis, an extracellular pH of 6.6 induced by metabolic acidosis (low HCO_3^-) or artificial buffers caused a small and much slower decline of left ventricular pressure development. Under the latter conditions, intracellular pH decreased much less than with respiratory acidosis. Studies with isolated cardiac sarcolemma showed that both high and low affinity Ca^{2+} binding was inhibited at pH 6.6 relative to pH 7.4. Verapamil inhibited only low affinity Ca^{2+} binding. From these and other data, it is concluded that increased extracellular H^+ in the presence of high pCO_2 causes a rapid fall of intracellular pH and exerts a negative inotropic effect primarily by competing with Ca^{2+} for intracellular calcium binding sites, although extracellular sites are also involved. It is proposed that H^+ interferes with that phase of the excitation-contraction coupling process whereby activator calcium under the control of the plateau phase of the action potential causes regenerative release of calcium from intracellular stores.

Hearts perfused with 5 mM glucose, 5 mM acetate and $5 \times 10^{-}$ units/ml of insulin were rapidly frozen different times up to 3 min after the pH 7.4 to 6.6 transition with respiratory acidosis, and analyzed for metabolic intermediates. At the half-time for decreased left ventricular pressure development (5 sec), ATP and creatine phosphate levels increased while ADP levels decreased. Subsequently, creatine phosphate and ATP levels decreased while ADP levels increased, indicating an imbalance between the rates of production and utilization of ATP. These data show that a fall in the rate of energy production is not responsible for the initial negative inotropic effect of H^+. Glycolytic flux, oxygen uptake and citric acid cycle activity decreased rapidly with pH 6.6 perfusion. Acetyl-CoA levels increased linearly during the first minute while oxalacetate and α-ketoglutarate levels rapidly decreased. Citrate, malate, aspartate and alanine levels initially increased and subsequently decreased, with malate and alanine levels finally rising again. Changes of glutamate levels were opposite to those of aspartate. Control of citric acid cycle activity is discussed in relation to the coordination of inhibitory interactions at the sites of citrate synthase, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase. During the transition state, flux through the different steps of the cycle is non-uniform due mainly to changes of tissue aspartate levels, but the predominant initial interactions appear to be mediated by increases of the NADH/NAD and ATP/ADP ratios.

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complicated by difficulties in maintaining a sharp pH transition in the fluid entering the coronary circulation when the net cardiac output fell to zero due to mixing effects in the fluid space between the aorta and the aortic reservoir Thus when the left ventricular output at pH 6 6 fell below the coronary perfusion rate buffer at a higher pH was introduced through the coronary circulation This produced a biphasic decrease of left ventricular pressure first to the aortic perfusion pressure which lasted from 30 to 60 sec followed by a further decline to the final steady-state level as illustrated in Fig. 1 A. In order to eliminate these difficulties the total left ventricular output was diverted through the air chamber and thence through the coronary circulation by placing a clamp above the aortic cannula The fluid volume of the air chamber was adjusted to be slightly greater than the stroke volume of the left ventricle in order to achieve a rapid exchange of fluid entering from the left atrial reservoir within 3



Fig. 1 Comparison of the kinetics of H inhibition in the standard working heart perfusion apparatus (A) and the closed aorta working heart perfusion apparatus (B) 1 A the rapid change of perfusate from pH 7 4 to 6 6 produces biphasic fall of left ventricular pressure The first transient steady-state level is produced by temporary increase of pH of the buffer perfusing the coronary essels caused by reversal of net flow n the fluid column abov the aortic cannula, while during the secondary fall perfusion at pH 6 6 is established 1 B rapid monotonic decrease of left ventricular pressure the first derivative of left ventricular pressure and aortic pressure is obtained due to the rapid clearance of pH 7 4 buffer by fluid at pH 6 6 Hearts were perfused with 5 mM glucose and 5 × 10 units/ml of insulin

to 4 beats. A major advantage of this closed aorta perfusion system for the present studies is that measurable net left ventricular fluid displacement is maintained over a wide range since the aortic perfusion pressure head is not fixed but is continuously determined by the left ventricular output and the coronary flow rate Furthermore diversion of the entire left ventricular output through the coronary arteries resulted in a high level of oxygenation of the myocardium above flow rates of about 4 ml/min with oxygen tensions in the coronary effluent characteristically above 150 mm Hg. With the closed aortic perfusion the decrease of left ventricular pressure development upon changing the perfusion fluid from pH 7 4 to 6 6 was rapid (t 1/2 about 5 sec) monotonic and reproducible (Fig. 1 B) At pH 7 4 total left ventricular output directly measured from the rate of coronary perfusion was 35 to 40 ml/min with an aortic pressure of about 165 cm H_2O which could be maintained for hours The perfusion fluid was normally not recirculated.

In order to evaluate quantitatively the effect of H on contractile performance of the heart the pH of fluid entering the ventricle via the atrial reservoir was progressively lowered after first returning perfusion to buffer at pH 7 4 prior to each successive change of pH in the second buffer (Fig. 2) The pH was altered by changing the CO_2 concentration in equilibrium with a constant bicarbonate concentration (25 mM) in accordance with the Henderson-Hasselbach equation. Although the extent of the inhibition of contractility was determined by the absolute pH of the second buffer the half-time to reach a new steady-state left ventricular pressure was constant (approximately 5 sec) A plot of the changes of first derivative of the left ventricular pressure against the pH of the fluid in the atrial reservoir is shown in Fig. 3 It is seen that the decrease of contractility was most rapid over the pH range from 7.2 to 6 9

The fall of contractility induced by lowering the pH may be compared with that obtained by addition of verapamil (10⁻⁶M) La^{3+} (10⁻⁵M) or transition to Ca^{2+} free perfusion (Fig 4) With each treatment, the decrease of left ventricular pressure was monotonic and the half-time was about 5 sec Verapamil and La are known to penetrate poorly across the intact sarcolemma, and are thought to exert their negative inotropic effects by interfering with entry of Ca^{2+} during the plateau phase of the action potential (6 22 4) Consequently it seemed plausible that the high external [H] was similarly interfering with Ca^{2+} at a rapidly exchangeable extracellular site hence decreasing the amount of Ca^{2+} available for troponin binding

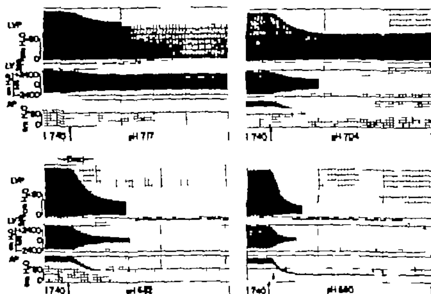


Fig. 2 Effect of decreased pH on left ventricular pressure (LVP), the first derivative of left ventricular pressure (LV dP/dt) and the aortic perfusion pressure (AP) with the closed aorta perfusion. The perfusion fluid contained 5 mM glucose 5 mM acetate 5 10^{-3} units/ml of insulin and 1.25 mM Ca^{2+}

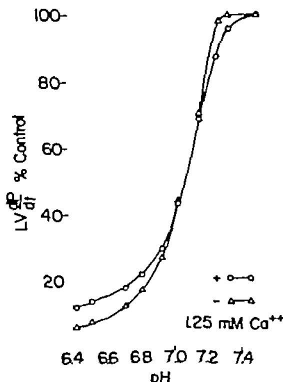


Fig. 3 Titration of perfusate pH with respect to left ventricular dP/dt. The perfusion fluid was the same as that of Fig. 2. Steady-state values were obtained within 30 sec at each pH and perfusion at pH 7.4 was restored until the initial level of contractility was observed before each transition to different pH

The possibility appears to be strengthened by the fact that just as the inhibition induced by verapamil is reversed by an increase of extracellular Ca^{2+} concentration so is the inhibition induced by increased H^+ reversed by Ca^{2+}. Thus Fig 5 shows that following a pH 7.4 to 6.6 transition contractility is rapidly restored by increasing the Ca^{2+} in the perfusate from 1.25 to 8 mM even after 10 min (Fig. 5 A) or 20 min (Fig. 5 B) perfusion at pH 6.6. Likewise when the pH and Ca^{2+} concentration of the second buffer were changed simultaneously the decrease of left ventricular pressure normally seen with the pH 7.4 to 6.6 transition was gradually abolished as the Ca^{2+} concentration was increased to 20 mM (Fig. 6). These experiments, therefore demonstrate the competitive nature of the H^+ and Ca^{2+} interaction on myocardial contractility.

The relationship between the percentage change of left ventricular pressure and the Ca^{2+} concentration in the perfusate at pH values of 7.40 6.77 and 6.50 is shown in Fig. 7. Sigmoid curves are obtained, and it is seen that the major effect of lowering the pH is to shift the normal [Ca^{2+}] of 0.6 to 0.8 mM for half maximal pressure development at pH 7.40 towards higher values.

Similar shifts of the Ca^{2+} titration curve against the percentage change of left ventricular pressure were obtained by addition of epinephrine or verapamil to hearts perfused with 1.25 mM Ca^{2+} at pH 7.4. Fig. 8 shows that whereas 0.5 μg/ml of epinephrine produced a 2-fold increase in sensitivity of the heart to extracellular Ca^{2+} 10^{-7}M verapamil increased

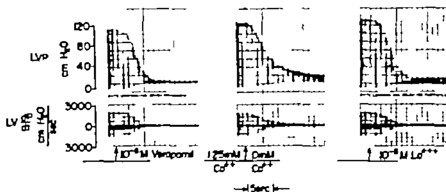


Fig. 4 Inhibition of left ventricular pressure (LVP) and the first derivative of left ventricular pressure (LV dP/dt) induced by 10^{-6} M verapamil, no calcium and 10^{-5} M La^{3+} with the closed aorta perfusion. The perfusion fluid was the same as that of Fig.

the Ca^{2+} concentration required for half-maximal peak left ventricular pressure from 0.6 to ...9 mM. The inhibitory effect of verapamil was partially overcome by epinephrine as seen by the lessened shift of the Ca^{2+} titration curve towards higher perfusate Ca^{2+} concentrations with simultaneous epinephrine and verapamil addition. Epinephrine also caused a shift of the Ca^{2+} titration curves obtained at acid pH towards lower Ca^{2+} concentrations (data not shown). Epinephrine increases the slow inward Ca^{2+} current of the action potential (6, 25) while verapamil (22) and H^+ (17) cause an inhibition. These effects tend to support the concept that competition with Ca^{2+} occurs at superficially located Ca^{2+} binding sites in the membrane. However further experiments described below indicate fundamental differences between the interactions of verapamil and H^+ at Ca^{2+} binding sites and suggest that increased extracellular $[H^+]$ has important intracellular effects which are directly involved in causing the initial decline of tension development.

Further insight into the molecular nature of the competition between Ca^{2+} and H^+ was gained by isolating and purifying guinea pig sarcolemma (26). Calcium binding to the purified sarcolemma fragments after zonal centrifugation to remove mitochondria was studied by incubating an aliquot of the membrane suspension (approximately 0.5 mg of protein) in Eppendorf centrifuge tubes in a final volume of 1 ml containing 10 mM Tris-Cl pH 7.4, Ca^{2+} concentrations in the range from 1-800 μM and $^{45}Ca^{2+}$ (10,000-50,000 cpm/ml). After a 5-min incubation at room temperature the mixture was centrifuged and an aliquot of the supernatant was removed for counting. The insides of the tubes were washed with 10 mM Tris-Cl, the membrane pellet solubilized with 0.5 ml of 8N formic acid and an aliquot removed into 5 ml of Hydromix fluid (York

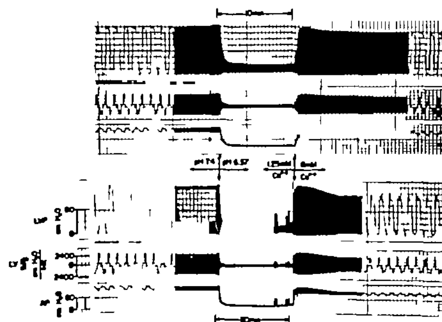


Fig. 5 Reversal of pH 6.57 inhibition of left ventricular pressure (LVP), it first derivative (LV dP/dt) and aortic perfusion pressure (AP) by increased perfusate Ca^{2+} concentration. The pH of the standard perfusion fluid was changed from 7.40 to 6.57 by increasing the pCO_2 from 36 to 288 mm Hg. After 10 min (top three traces) or 70 min (bottom three traces) perfusion at pH 6.57 the perfusate $[Ca^{2+}]$ was increased from 1.25 to 8 mM. The perfusion fluid was the same as that of Fig. ..

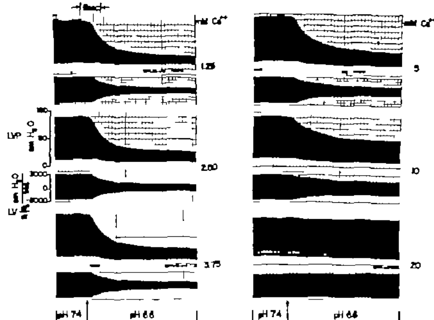


Fig. 6. Effect of increasing Ca^{2+} concentration on the decrease of left ventricular pressure (LVP) and the first derivative of left ventricular pressure (LV dP/dt) produced by decreasing the pH from 7.4 to 6.6. Note that the $[H^+]$ and $[Ca^{2+}]$ were changed simultaneously. *Hearts were perfused with* closed aorta with the same basic medium as in Fig. 2.

town Research New York N Y) for counting. Calcium binding to the membranes was calculated from the specific activity of the added $^{45}Ca^{2+}$ and the counts retained by the membranes. The results of a representative experiment expressed in the form of a Scatchard plot are presented in Fig. 9 The curve shows no sharp break, *indicating that there* may be more than two types of Ca^{2+} binding sites However from extrapolations of the linear portions of the curve at the extremes of low and high amounts of bound Ca^{2+} values for two classes of binding sites may be calculated. The extrapolated intercept in the abscissa (n) represents the number of binding sites per mg of membrane protein, while the intercept on the ordinate is nK where K is the association constant The data from this experiment show that the number of high Ca^{2+} affinity sites was about 10 times smaller than the number of low Ca^{2+} affinity sites. The apparent K_m of the low affinity *sites (0.8 mM) is similar to the overall K_m for Ca^{2+}*

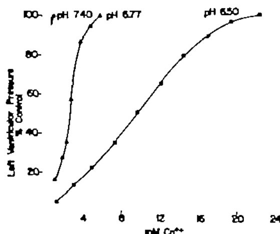


Fig. 7 Titration of left ventricular pressure changes with respect to the $[Ca^{2+}]$ of the perfusion fluid at pH 7.40, 6.77 and 6.50 Hearts were perfused as described in Fig. 2.

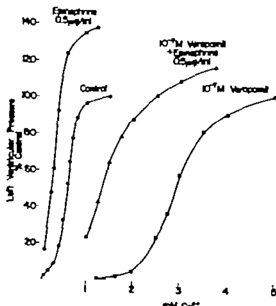


Fig. 8. Titration of left ventricular pressure changes with respect to the $[Ca^{2+}]$ of the perfusion fluid in control heart or hearts perfused with 0.5 μg/ml epinephrine, 10^{-7} M verapamil, and 0.5 μg/ml epinephrine plus 10^{-7} M verapamil. Hearts were perfused as described in Fig. 2.

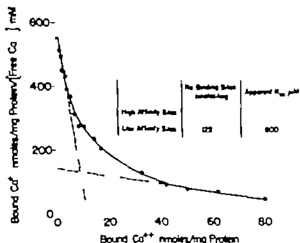


Fig. 9 Scatchard plot of Ca^{2+} binding to guinea pig cardiac sarcolemma.

required to increase left ventricular pressure development (cf Figs. 7 8) hence these sites may represent specific Ca^{2+} binding sites for Ca entry on the outer surface of the sarcolemma. This suggestion is supported by the specific inhibition of only low affinity calcium binding by verapamil (Fig. 10) On the other hand high affinity sites may represent Ca^{2+} binding sites on the inside surface of the sarcolemma related specifically to mechanisms involved in secondary intracellular calcium release or to Ca^{2+} efflux from the cell The apparent Km of 16 μM is high for sites interacting with intracellular Ca^{2+} but this value (unlike that for the low affinity apparent Km) was more variable between different preparations (range 1 70 μM) The presence of a small number of very high affinity sites (?7) cannot be excluded since Ca binding was not studied below 10 M A comparison of Ca binding to the sarcolemma at pH 7 4 and 6 6 (Fig. 10) shows that in contrast to verapamil increased [H] inhibited both high and low affinity calcium binding. It would appear therefore that the effects of decreased pH on the affinity of sarcolemma membranes for Ca^{2+} are not restricted to sites on the surface normally facing outwards in the intact sarcolemma but also involve internally facing sites

Further evidence of both extra- and intracellular H -sensitive sites in the intact heart is obtained from a comparison of the effects of metabolic (low HCO_3^-) and respiratory (high pCO_2) acidosis on mechanical performance Fig. 11 shows that although the extracellular pH was decreased from 7.4 to 6 6 under both conditions the heart was much more sensitive to the fall of pH induced by respiratory than by metabolic acidosis. A comparison of titrations of left ventricular pressure changes with pH in hearts using MOPS (morpholino propane sulfonic acid) to replace the normal bicarbonate buffer is shown in Fig. 1 A typical sigmoidal increase of left ventricular pressure with increasing pH is observed with the bicarbonate-CO_2 buffer (cf Fig. 3) while with 10 mM MOPS as the major buffering anion (together with the normal phosphate concentration present in Krebs bicarbonate medium) the fall of left ventricular pressure with decreasing pH was much less severe When 10 mM MOPS was added to the bicarbonate buffer only a slight protection against the effects of low pH was obtained. These data indicate that the major sites responsible for the Ca^{2+} H interaction are readily accessible to CO_2 but not to H *per se*

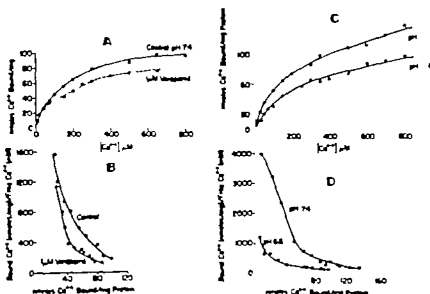


Fig. 10 Effect of 10^{-6} M verapamil and pH 6 6 on Ca^{2+} binding to guinea pig cardiac sarcolemma. The top curves show the amount of Ca^{2+} bound as function of the free Ca^{2+} concentration while Scatchard plot are shown in the bottom curves.

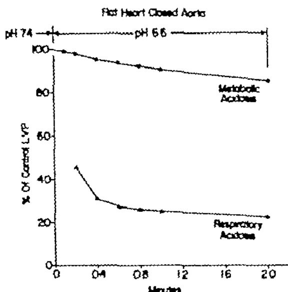


Fig. 11 Comparison of left ventricular pressure changes (LVP) induced by metabolic and respiratory acidosis (pH 6.6). Metabolic acidosis was produced by addition of *HCl* and respiratory acidosis by an increase of pCO_2. Hearts were perfused with closed aorta with fluid containing 5 mM glucose 10^{-2} units/ml of insulin and 1.5 mM Ca^{2+}

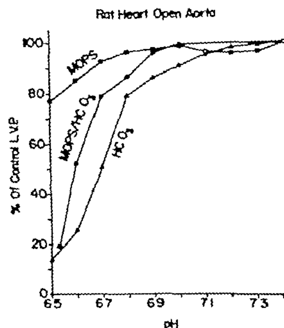


Fig. 1 Titration of changes of left ventricular pressure (LVP) against pH in hearts perfused with 25 mM bicarbonate or with the bicarbonate replaced by 10 mM MOPS (morpholinopropane sulfonic acid) using the standard working heart apparatus. The perfusion fluid contained 5 mM glucose 10 units/ml of insulin and 1.5 mM Ca^{2+}

The left ventricular pressure development of hearts perfused at pH 7.4 with a mixture of artificial buffers replacing bicarbonate responds normally to an increased Ca^{2+} concentration in the perfusion fluid (Fig. 13). However, as shown in Fig. 13 very little displacement of the Ca^{2+} titration curves towards increased Ca^{2+} concentration is observed upon decreasing the pH of the buffer mixture. The Ca^{2+} titration curve for bicarbonate buffer at pH 6.8 is included in Fig. 13 for comparison with the results obtained with the artificial buffer mixture at pH 7.4, 6.8 and 6.6.

It is to be expected that the higher permeability of the plasma membrane to CO_2 relative to bicarbonate or the arterial buffers will cause the intracellular pH to respond more rapidly to a lowering of the extracellular pH during respiratory acidosis than with metabolic acidosis or with poorly penetrating artificial buffers at low pH (28). This effect has in fact been observed in an experiment similar to that shown in Fig. 11 (unpublished observations). The above data, taken together, are best interpreted in terms of competition between Ca^{2+} and H^+ at both extracellular and intracellular sites with the intra-

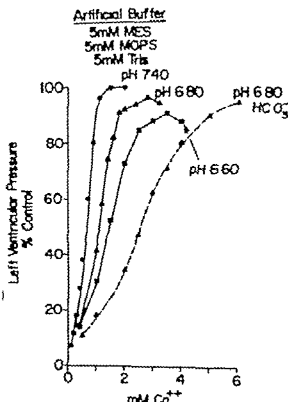


Fig. 13 Titration of left ventricular pressure changes against $[Ca^{2+}]$ of the perfusion fluid in heart perfused with closed aorta at varied pH values with the artificial buffer mixture and pH 7.4, 6.8 or 6.6 and bicarbonate CO_2 buffer at pH 6.8.

cellular sites having a quantitatively greater effect on contractile function (29-34) Presumably the sarcolemma phospholipid membrane provides the permeability barrier to transport of H bicarbonate and artificial buffers and hence separates the intracellular from the extracellular space However in cardiac muscle the invaginations of the plasma membrane made by the T-tubules complicate the interpretation of experimental results particularly in relation to an anatomical basis for cellular calcium pools and binding sites (16 35) Thus externally added agents may not have equal accessibility to all Ca binding sites on the sarcolemma while conversely concentrations of ions in the lumen of the T tubules may be different from those in the bulk phase of the extracellular space Unlike cardiac muscle skeletal muscle does not respond to respiratory acidosis with a decreased tension development (36). This effect may be connected with the fact that contraction of skeletal muscle is insensitive to external Ca^2 concentration, and suggests that the primary action of H should be on some property specific to cardiac muscle probably related to the excitation-contraction coupling mechanism. A satisfactory explanation for the mechanism of action of H on the contraction of cardiac muscle is limited by lack of knowledge of the precise relationship of the Ca^2 influx during the slow phase of the action potential to release of Ca^2 from internal storage sites. In fact, even the ultramicroscopic identification of the Ca^2 storage sites is in doubt in cardiac muscle and may be species dependent By analogy with skeletal muscle the major storage sites for release of activator Ca is generally assumed to be the sarcoplasmic reticulum in mammalian cardiac muscle although a subsarcolemma location is more likely in amphibian and neonatal cardiac muscle due to the poor development of the sarcoplasmic reticulum in these species (5) In skeletal muscle it is considered more likely that depolarization of the sarcoplasmic reticulum membrane provides the stimulus for Ca release rather than a direct Ca^2 trigger mechanism induced by Ca^2 flux during the action potential (37) On the other hand Ca^2 influx across the sarcolemma is quantitatively much greater in cardiac muscle so that the Ca^2 trigger mecham m for regenerative Ca^2 release may predominate in cardiac muscle Such regenerative Ca^2 release induced by a small increase of Ca^2 release caused by a mall increase of Ca^2 concentration from 5 to 8 × 10 M has been demonstrated in skinned cell from mammalian (but not frog) cardiac muscle (38) In a kinetic study of $^{45}Ca^2$ washout from the perfused interventricular septum of rabbit heart Poole Wilson and Langer (18) found that acidosi induced by high pCO_2 did not affect Ca^2 flux from the most rapidly exchanging compartment which is indistinguishable from that of the extracellular fluid but decreased both $^{45}Ca^2$ influx and efflux from a more slowly exchanging compartment tentatively identified from other studies as the sarcoplasmic reticulum (35) The amount of Ca^2 in this pool may be directly pH dependent (15) but an alternative mechanism which accounts for all the known facts may be suggested which envisions the primary site of H interaction as intracellular between the Ca^2 trigger mechanism and the secondary release of Ca^2 from the sarcoplasmic reticulum In this model (cf 18) extracellular H diminishes Ca^2 influx during the plateau phase of the cardiac action potential (17) but a change of intracellular pH is also required to produce a decreased release of intracellularly sequestered Ca^2 Hence a decreased availability of Ca^2 for binding to troponin results with consequent decrease of tension development.

Energetics of the pH 7 4 to 6 6 transition The work presented above has focused attention on the dynamics and mechanism of inhibition of left ventricular pressure development induced by acidosis in the intact heart Further studies have been concerned with the nature of the interactions whereby the rate of energy production is coordinated with the rate of energy utilization For this purpose the closed aorta working perfused preparation shown in Fig. 1 B was used with 5 mM glucose 5 mM acetate and 5 × 10 units/ml of insulin added to Krebs bicarbonate medium containing 1.5 mM Ca^2 Acidosis was induced by increasing the percentage of CO_2 in the equilibrating gas mixture from 5 to 35 % During the pH transition (cf Fig. 1 B) diastolic left ventricular pressure decreased within 30 sec from 170 to 40 cm H_2O while aortic pressure decreased from 165 to 36 cm H_2O with a half time of 5 sec Oxygen consumption fell from 11 17 to a stable value of 3 06 matoms/g dry wt/hr during the first 3 min while the rate of coronary perfusion fell from 26 to 4 ml/min The PO_2 of the effluent fluid from the heart remained above 80 mm Hg at all times. Glycolytic flux measured as the release of tritium from [3-³H] glucose decreased from 736 to a minimum of 139 µmoles/g dry wt/hr after 60 sec subsequently recovering to 217 µmoles/g dry wt/hr by 180 sec Lactate production also declined from 168 to a minimum of 85 µmoles/g dry wt/hr at 60 sec then increased to 382 µmoles/g dry wt/hr by 180 sec The lactate/pyruvate ratio in the perfusion fluid increased from 2 to 35 4 after 3 min. Under control conditions at pH 7 4 acetate oxidation accounted for 35 % of the oxygen consumption.

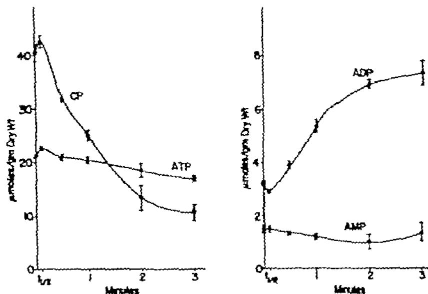


Fig. 14 Changes in the levels of adenine nucleotides and creatine phosphate in hearts rapidly frozen different times after the transition from perfusion at pH 7 4 to 6 6 The time indicated at t 1/2 is 5 sec and corresponds to the point at which the decrease of left ventricular pressure is half-maximal. Hearts were perfused with closed aorta with 5 mM glucose, 5 mM acetate and 5 × 10⁻³ units/ml of insulin. Values shown are means ± SEM of 4 to 8 hearts.

Fig. 14 shows changes of the contents of adenine nucleotides and creatine phosphate in hearts during the first 3 min after the pH 7 4 to 6 6 transition A small but statistically significant decrease of ADP ($p < 0.05$) and increase of ATP ($p < 0.01$) were found at the half-time of the decrease of contractility (5 sec) Mean creatine phosphate levels also increased but this change was not statistically significant. Although ATP and creatine phosphate levels subsequently decreased from the control to new lower steady state levels while ADP levels increased these changes occurred much later than the immediate fall of contractile force These results therefore demonstrate that the initial fall of contractility induced by acidosis is not connected with any mechanism involving failure of energy production. However an imbalance between the rates of energy production and utilization manifests itself after the first 5 sec following the pH transition, suggesting that decreased energy production may limit mechanical performance under steady state conditions of acidosis.

Changes of citric acid cycle intermediates and amino acid level in hearts immediately after the pH 7 4 to 6 6 transition are shown in Figs 15 and 16. During the first 30 sec citrate malate and acetyl-CoA levels increased while α-ketoglutarate levels decreased Subsequently citrate levels decreased while acetyl-CoA levels continued to rise reaching a steady state after 1 min Aspartate levels increased during the first minute while glutamate levels showed a reciprocal fall (Fig. 16) The pool size of total citric acid cycle intermediates only varied from 6 1 3 6 μmoles/g dry wt despite a 70 % decrease of flux through the citric acid cycle

The alanine content showed no statistically significant changes during the first two minutes but increased sharply from the second to the third minute There was thus a poor correlation between changes of the pool size of citric acid cycle intermediates and the alanine content suggesting that mechanisms other than double transamination between alanine and aspartate aminotransferase are involved in the regulation of the total tissue contents of cycle intermediates under these conditions (21 39) The alternative mechanism for net interconversion between 3-carbon and 4-carbon intracellular carboxylic acids probably involves activity of malic enzyme (39).

Of greater significance are the factors involved in the regulation of citric acid cycle flux during the acidotic transition. Overall citric acid cycle flux calculated on the basis of substrate utilization and oxygen consumption (21) decreased rapidly during the first minute and reached a new steady state within 3 min after the pH transition However from the changes of the pool sizes of the intermediates it is evident that during the transition phase flux is not decreased uniformly through each of the individual steps of the citric acid cycle A full discussion of the various factors influencing flux through the citric acid cycle is beyond the scope of this paper but the most important interactions thought to be operating during the pH transition will be summarized. The rate of glucose conversion to acetyl-CoA via glycolysis falls rapidly due to H inhibition of phosphofructokinase which increases the apparent Km for fructose-6-P (40 41) to inhibition of the rate of transfer of reducing equivalents into mitochondria (42) and probably also to a decrease in the propor

cellular sites having a quantitatively greater effect on contractile function (29-34) Presumably the sarcolemma phospholipid membrane provides the permeability barrier to transport of H bicarbonate and artificial buffers and hence separates the intracellular from the extracellular space However in cardiac muscle the invaginations of the plasma membrane made by the T-tubules complicate the interpretation of experimental results particularly in relation to an anatomical basis for cellular calcium pools and binding sites (16 35) Thus, externally added agents may not ha e equal accessibility to all Ca binding sites on the sarcolemma while conversely concentrations of ions in the lumen of the T-tubules may be different from those in the bulk phase of the extracellular space Unlike cardiac muscle skeletal muscle does not respond to respiratory acidosis with a decreased tension development (36) This effect may be connected with the fact that contraction of skeletal muscle is insensitive to external Ca^2 concentration and suggests that the primary action of H should be on some property specific to cardiac muscle probably related to the excitation-contraction coupling mechanism. A satisfactory explanation for the mechanism of action of H on the contraction of cardiac muscle is limited by lack of knowledge of the precise relationship of the Ca^2 influx during the slow phase of the action potential to release of Ca from internal storage sites In fact even the ultramicroscopic identification of the Ca^2 storage sites is in doubt in cardiac muscle and may be species dependent. By analogy with skeletal muscle the major storage sites for release of activator Ca is generally assumed to be the sarcoplasmic reticulum in mammalian cardiac muscle although a subsarcolemma location is more likely in amphibian and neonatal cardiac muscle due to the poor development of the sarcopla mic reticulum in these species (5) In skeletal muscle it is considered more likely that depolarization of the sarcoplasmic reticulum membrane provides the timulus for Ca release rather than a direct Ca trigger mechanism induced by Ca^2 flux during the action potential (37) On the other hand, Ca^2 influx acros the sarcolemma is quantitati ely much greater n cardiac muscle so that the Ca trigger mechanism for regenerative Ca^2 release may predominate n cardiac muscle Such regenerative Ca release induced by a small increase of Ca^2 release caused by mall increase of Ca^2 concentration from 5 to 8 10 M ha been demonstrated in skinned cell from mammalian (but not frog) cardiac muscle (38) In kinetic study of $^{45}Ca^2$ washout from the perfused interventricular septum of rabbit heart Poole Wilson and Langer (18) found that acidosis induced by high pCO_2 did not affect Ca^2 flux from the most rapidly exchanging compartment which is indistinguishable from that of the extracellular fluid but decreased both $^{45}Ca^2$ influx and efflux from a more slowly exchanging compartment tentatively identified from other studies as the sarcoplasmic reticulum (35) The amount of Ca^2 in this pool may be directly pH dependent (15) but an alternati e mechanism which accounts for all the known facts may be suggested which envisions the primary site of H interaction as intracellular between the Ca^2 trigger mechanism and the secondary release of Ca^2 from the sarcoplasmic reticulum. In this model (cf 18) extracellular H diminishes Ca^2 influx during the plateau phase of the cardiac action potential (17) but a change of intracellular pH is also required to produce a decreased release of intracellularly sequestered Ca^2 Hence a decreased availability of Ca^2 for binding to troponin results with consequent decrease of tension development.

Energetics of the pH 7 4 to 6 6 transition The work presented above has focused attention on the dynamics and mechanism of inhibition of left ventricular pressure development induced by acidosis in the intact heart. Further studies have been concerned with the nature of the interactions whereby the rate of energy production is coordinated with the rate of energy utilization For this purpose the closed aorta working perfused preparation shown in Fig. 1 B was used with 5 mM glucose 5 mM acetate and 5×10^{3} units/ml of insulin added to Krebs bicarbonate medium containing 1..5 mM Ca^2 Acidosis was induced by increasing the percentage of CO_2 in the equilibrating gas mixture from 5 to 35 % During the pH transition (cf Fig. 1 B) diastolic left ventricular pressure decreased within 30 sec from 170 to 40 cm H_2O while aortic pressure decreased from 165 to 36 cm H_2O with a half-time of 5 sec Oxygen consumption fell from 11 17 to a stable value of 3.06 matoms/g dry wt/hr during the first 3 min while the rate of coronary perfusion fell from 26 to 4 ml/min. The PO_2 of the effluent fluid from the heart remained above 80 mm Hg at all times. Glycolytic flux measured as the release of tritium from [3-³H] glucose decreased from 736 to a minimum of 139 μmoles/g dry wt/hr after 60 sec subsequently recovering to 217 μmoles/g dry wt/hr by 180 sec Lactate production also declined from 168 to a minimum of 85 μmoles/g dry wt/hr at 60 sec then increased to 382 μmoles/g dry wt/hr by 180 sec The lactate/pyruvate ratio in the perfusion fluid increased from to 35 4 after 3 min. Under control conditions at pH 7 4 acetate oxidation accounted for 35 % of the oxygen consumption

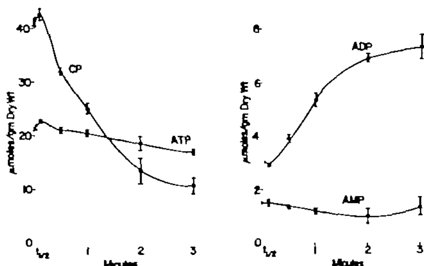


Fig. 14 Changes in the levels of adenine nucleotides and creatine phosphate in hearts rapidly frozen different times after the transition from perfusion at pH 7 4 to 6 6. The time indicated at t 1/2 is 5 sec and corresponds to the point at which the decrease of left ventricular pressure is half-maximal. Hearts were perfused with closed aorta with 5 mM glucose 5 mM acetate and 5 × 10⁻³ units/ml of insulin. Values show are means ± SEM of 4 to 8 hearts.

Fig. 14 shows changes of the contents of adenine nucleotides and creatine phosphate in hearts during the first 3 min after the pH 7 4 to 6 6 transition. A small but statistically significant decrease of ADP ($p < 0.05$) and increase of ATP ($p < 0.01$) were found at the half-time of the decrease of contractility (5 sec). Mean creatine phosphate levels also increased but this change was not statistically significant. Although ATP and creatine phosphate levels subsequently decreased from the control to new lower steady state levels while ADP levels increased, these changes occurred much later than the immediate fall of contractile force These results therefore demonstrate that the initial fall of contractility induced by acidosis is not connected with any mechanism involving failure of energy production. However an imbalance between the rates of energy production and utilization manifests itself after the first 5 sec following the pH transition, suggesting that decreased energy production may limit mechanical performance under steady state conditions of acidosis.

Changes of citric acid cycle intermediates and amino acid levels in hearts immediately after the pH 7 4 to 6.6 transition are shown in Figs. 15 and 16. During the first 30 sec citrate malate and acetyl-CoA levels increased while α-ketoglutarate levels decreased. Subsequently citrate levels decreased while acetyl-CoA levels continued to rise reaching a steady state after 1 min. Aspartate levels increased during the first minute while glutamate levels showed a reciprocal fall (Fig. 16). The pool size of total citric acid cycle intermediates only varied from 6 to 3 6 µmoles/g dry wt despite a 70 % decrease of flux through the citric acid cycle The alanine content showed no statistically significant changes during the first two minutes but increased sharply from the second to the third minute. There was thus a poor correlation between changes of the pool size of citric acid cycle intermediates and the alanine content, suggesting that mechanisms other than double transamination between alanine and aspartate aminotransferase are involved in the regulation of the total tissue contents of cycle intermediates under these conditions (?1 39) The alternative mechanism for net interconversion between 3-carbon and 4-carbon intracellular carboxylic acids probably involves activity of malic enzyme (39).

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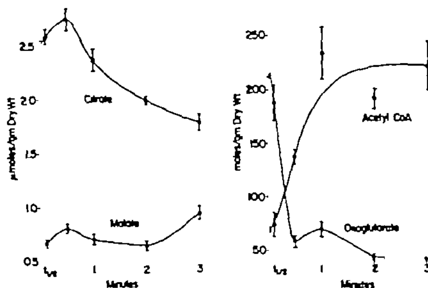


Fig. 15. Changes in the levels of citrate, malate, acetyl-CoA and α-ketoglutarate (oxoglutarate) in heart frozen different times after the transition from pH 7.4 to 6.6. The perfusion conditions were the same as those of Fig. 14

tion of pyruvate dehydrogenase in the active non phosphorylated form (43). Acetate conversion to acetyl-CoA although inhibited decreases less than its rate of utilization by citrate synthase with the result that acetyl-CoA levels increase (Fig. 15) and in the steady state account for about 50 % of the total CoA content of the heart (21, 44). Oxalacetate level were not measured directly in this experiment but values calculated by assuming equilibrium of aspartate aminotransferase indicated a fall from about 20 to 7 nmoles/g dry wt during the first 30 sec. This change is associated with an increase of the malate/oxalacetate ratio and decreased flux of aspartate to oxalacetate as indicated from the increase of aspartate content (Fig. 16)

Citrate synthase is inhibited both by a direct effect of H on the enzyme which increases the apparent K_m for oxalacetate (45) as well as by an absolute fall of oxalacetate concentration assuming that the change in the mitochondrial compartment reflects that in the total tissue. Nevertheless total tissue citrate levels initially increase slightly rather than fall as expected from an inhibition of flux through citrate synthase. With acetate present as substrate in addition to glucose the total citrate pool is very large and may not accurately reflect changes in the mitochondrial compartment, particularly as the tricarboxylate translocator has a low activity in heart muscle (46). However it is known that the citrate level is sensitively regulated by the coordination of interactions at citrate synthase and NAD-linked isocitrate dehydrogenase (47) and if the observed changes of tissue citrate content are not entirely confined to the cytosolic compartment

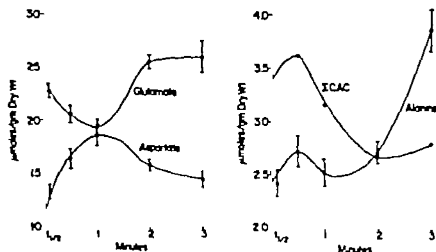


Fig. 16. Changes in the levels of glutamat, aspartate, alanine and total citric acid cycle intermediates (Σ CAC) in heart frozen different times after the transition from pH 7.4 to 6.6. The perfusion condition are the same as those of Fig. 14. The sum of citric acid cycle intermediates at each time point w obtained from the measured content of citrate, isocitrate, α-ketoglutarate and malat

it must be presumed that initially citrate synthase is less inhibited than isocitrate dehydrogenase. When tissue citrate levels fall after 30 sec it may be assumed that flux through isocitrate dehydrogenase is then slightly greater than that through citrate synthase. These minor modulations of enzyme activities relative to the overall flux decrease are probably associated with changes in the relative strengths of interactions of ADP, NADH and H^+ at isocitrate dehydrogenase (48, 49). Thus, an increased energy state associated with increased mitochondrial ATP/ADP and NADH/NAD ratios (state 3 to 4 transition) caused by decreased cardiac work (50) may initially be the dominant factor causing inhibition of flux through citrate synthase and isocitrate dehydrogenase, with additional effects due to increased $[H^+]$ and a decreased ATP/ADP ratio becoming important at a slightly later time. It is, in fact, to be expected that the effects of increased extracellular $[H^+]$ will be first observed in enzymes in the cytosol, while the extra buffering capacity of the mitochondria will diminish and possibly retard the effects of the lowered cytosolic pH on the mitochondrial matrix pH (51).

The large rapid fall of α-ketoglutarate immediately after the pH transition (Fig. 15) indicates that during the first 30 sec, flux through α-ketoglutarate dehydrogenase is greater than that through isocitrate dehydrogenase. When the malate-aspartate cycle is operating for the transfer of reducing equivalents into mitochondria, intramitochondrial α-ketoglutarate is produced as a product of aspartate aminotransferase activity as well as that of isocitrate dehydrogenase (21). When flux through cytosolic and mitochondrial aspartate aminotransferase isoenzymes are equal, α-ketoglutarate formed in the mitochondria is stoichiometrically transported out into the cytosol and tissue contents of the malate-aspartate cycle intermediates remain constant. However, any changes of aspartate and glutamate contents in the tissue are associated with different flux rates of the transaminase reactions on either side of the mitochondrial membrane and because of the relatively large pool sizes of these amino acids, any rapid change of the aspartate content by its effect on α-ketoglutarate flux will be the dominant factor causing unspanning of cycle flux, as demonstrated earlier (21). The fall of glutamate and rise of aspartate depicted in Fig. 16 is interpreted in terms of a smaller decrease of flux through the glutamate-aspartate than that through the malate α-ketoglutarate translocator, with the consequence that flux through mitochondrial aspartate aminotransferase is greater than that through the cytosolic isoenzyme. α-Ketoglutarate dehydrogenase itself remains relatively activated, so that flux through this step is greater than the preceding step in the citric acid cycle by an amount equal to the rate of decrease of tissue α-ketoglutarate plus the rate of increase of tissue aspartate. Thus, a slight lack of coordination between inhibition of isocitrate dehydrogenase and α-ketoglutarate dehydrogenase results in a depletion of tissue α-ketoglutarate with a consequent rise of the aspartate/glutamate ratio. This in turn inhibits glutamate entry into the mitochondria since extramitochondrial aspartate is a competitive inhibitor of the glutamate-aspartate exchange (5?), with the result that the rate of generation of α-ketoglutarate is decreased. Flux through α-ketoglutarate dehydrogenase is thus decreased due to the lowered mitochondrial α-ketoglutarate concentration. A similar increase of tissue aspartate and fall of tissue glutamate has been observed during the transition from high to low work at pH 7.4 (50, 53). After the first minute following the pH transition aspartate levels fall while glutamate levels rise, and flux through α-ketoglutarate dehydrogenase becomes less than that through isocitrate dehydrogenase. These changes reflect an increased degree of inhibition at the α-ketoglutarate dehydrogenase step due to an increase of the mitochondrial NADH/NAD ratio, increased $[H^+]$, and possibly also to a direct inhibition of $[H^+]$ on the glutamate-aspartate translocator (5?), which further inhibits glutamate entry and production of mitochondrial α-ketoglutarate and aspartate via aspartate aminotransferase. However, this latter effect is probably of less importance when acetate or fatty acids are also present as substrates than when glucose is the only exogenous fuel (4).

Metabolic changes associated with Ca^{2+} reversal of the inhibition of cardiac contractility induced by acidosis. The fact that the inhibition of cardiac contractility induced by respiratory acidosis can be reversed by increasing the Ca^{2+} concentration in the perfusion medium has already been demonstrated in Fig. 5. The duration of the recovery period is dependent on the nature of the substrate supplied to the heart. In Fig. 17A, the heart was perfused for 15 min using the closed aorta technique with 5 mM glucose, 5 mM acetate and 5 × 10 units/ml of insulin at pH 7.4 before transfer ring to a second buffer equilibrated at pH 6.6. After 3 min of perfusion under acidotic conditions, 10 mM Ca^{2+} was added, and it is seen that complete recovery of systolic and diastolic left ventricular pressure was obtained. This could be sustained for at least 30 min. In Fig. 17 B the results of a similar experiment are shown but with acetate omitted from the perfusion medium. It is seen that the decrease of left

followed by reperfusion where the myofibrillar pattern is characteristic of the contracted state (57). However further work is needed to ascertain the nature of the biochemical link between loss of a critical rate of ATP generation and the changes associated with inability of metabolism and contraction to be restored. It may be speculated that maintenance of a critical low intracellular pH for a certain period of time either directly or indirectly causes irreversible damage to the mitochondria resulting in a deficiency in their ability to perform oxidative phosphorylation. Sites of interaction may be at the locus of specific anion translocators in the mitochondrial membrane e g. aspartate (47) ADP (58) or palmityl carnitine (59) or more generally on the permeability of the inner mitochondrial membrane resulting in the loss of cofactors such as CoA NAD adenine nucleotides (60 61) or directly on the energy conservation *mechanism (6., 63) Changes in calcium homeostasis* of the cell are probably intimately involved in the etiology of irreversible tissue damage but again it is not known whether this involves a primary effect on the permeability of the plasma membrane to ions including Ca^{2} to defective sequestration of Ca by the sarcoplasmic reticulum (65) or to an abnormally high mitochondrial calcium content (66 67) The model of heart failure illustrated in Fig. 17 would appear to offer a valuable system for elucidation of these uncertainties.

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DISCUSSION

Dr Morgan

Are there questions or comments on Dr Williamson s paper?

Dr Fitzgerald

Do you know what the lowest pH reached is in experimental ischemia and how does it compare with the pH achieved in your experiments Secondly have you happened to compare the effects on contractility of a beta-blocking drug at the various levels of pH Do beta-blockers in fact accentuate the reduction in contractility in the pH range 7 5-6 6? Theoretically they should but I have never actually seen any data which show that they do

Dr Williamson

To answer the last question first I have not tried propranolol but this would be interest ng. We have done the reverse namely to add catecholamines and that of course counteracts the decrease in contractility that occurs with acid pH I think Dr Neely will agree that it is extremely difficult to measure intracellular pH when coronary flow is very low The pHs of arterial and venous perfusates are different and the interarterial pH must be something in between. To use the DMO technique one must know the extracellular pH and in low flow ex

tained for ten to twenty minutes whereas in the ischemic preparation that Dr Neely works with half of the total adenine nucleotide pool is lost in the same period of time Dr Williamson s preparation is reversible at lea t by restoration of pH or by adding calcium, whereas after thirty minutes of severe ischemia in Dr Neely s model deterioration of function is not reversible I wonder whether break down of adenine nucleotides accounted for the irreversibility

Dr Williamson

The point I want to make is that loss of cellular nucleotides can be fantastically rapid In the last series of experiments I showed with glucose and insulin alone following calcium reversal to maximum contractility spontaneous failure occurred in less than five minutes and two thirds of a nucleotide pool had disappeared in that time I do not know which enzymes are activated to trigger nucleotide degradation. Is there any correlation between loss of adenine nucleotides and los of CPK or electrocardiographic changes in other models such as coronary artery ligation

Dr Sobel

The iew that ATP stores *per se* can be used as index of irreversibility is a problem. In some studies performed with Dr Maroko and others we examined ATP content in regions of ischemia identified first by an ST-mapping technique like the one described this morning erified subsequently both histologically and enzymatically The surprising result was that in the dog model the decline of total tissue ATP in a region of a frankly ischemic zone was ery slow – occurring over hours Furthermore with regard to protection with glucose-insulin- and potassium, in the same preparation the fall of ATP content is reversed in a zone of no ischemia The glucose actually does get into the cell since radioactively labeled glucose appears in glycogen even though most of the flu is glycogenolytic Thus glucose gains access to intracellular water and also raises the ATP content substantially toward normal in zones of severe schemia Unfortunately however we do not have evidence to suggest that the ATP content is closely related as an overall pool to the survival of ll in the region Since ATP is but one of the adenine nucleotides I hould think that even considering all of them only very loose coupling would be provided between cell death and what has happened to these particular moieties.

Dr Morgan

Dr Williamson, do you have any additional comment

Dr Williamson

The data I showed was selected to demonstrate two points 1) the decrease in contractility with acid pH is not due to a defect of energy metabolism because in the first few seconds when contractility decreases rapidly energy levels increase and) contractility was essentially independent of the creatine-phosphate or ATP level. I chose to look at acid pH rather than ischemia because it is important to define the sequence of biochemical events that occur I wanted to look at a rapid transition which one could manipulate I do think that the transitions that occur with acid pH have some relevance to true ischemia.

Dr Jennings

Dr Williamson s discussion of the role of calcium in ischemia i an important one I did not include Ca in the general list of the causes of irreversibility that I presented a few minutes ago because I believe that changes in its flux may be secondary events and because there may not be enough Ca^{2+} in areas of severe permanent ischemia for it to play a significant role There is little doubt that calcium ion is involved in cell death *in vivo* whenever cells die in the presence of an adequate arterial flow Earlier today I showed that contraction bands and mitochondrial Ca phosphate accumulation both occur in irreversibly injured cells if they are reperfused with arterial blood Although proved I believe that the contraction bands probably result from excess intracellular calcium The mitochondrial Ca obviously involves increased availability of sarcoplasmic Ca Furthermore the accumulation of ma sive amounts of calcium in the mitochondria is probably deleterious to cell function Lehninger has shown that Ca accumulation takes preference to oxidative phosphorylation whenever cations such as Ca are present in abundance adjacent to oxygenated mitochondria. Thus mitochondrial calcium accumulation should be a sociated with depletion of the net ATP a ailable to the cell Also the function of mitochondria massively loaded with Ca^{2+} probably i impaired Together these observations suggest Ca may be important when pla ma Ca^{2+} is available to the injured cells.

I have more difficulty finding a role for calcium in an area of severe permanent ischemia. In severe ischemia arterial collateral flows range from 0 to < 10 per cent of control flow Under such circum-

stances the amount of the available plasma calcium for exchange is low and the cell only is seeing that quantity being provided by the collateral flow plus the small amount of calcium available in the extracellular fluid trapped in the ischemic focus. Moreover Ca^{2+} cannot be actively accumulated in the mitochondria because of low ATP and the lack of O_2. Whether the quantity of calcium available is adequate to induce changes in the severely ischemic cell of the type which leads to the irreversible state remains unknown. If it is important it is noteworthy that Ca^{2+} does not induce in areas of severe ischemia either the contraction bands and mitochondrial changes seen in cells dying in the presence of arterial flow.

I think that a brief comment about tissue ATP levels is indicated. It is important to recognize that low tissue ATP is not invariably associated with cell death. For example Emmanuel Farber and his associates in Philadelphia have shown that ethionine administration is followed by accumulation of extraordinary amounts of fat in the liver cells. The ATP of this tissue is less than 10 per cent of control and remains at these low levels for a significant period of time. However cell death does not occur in this system. One can draw the conclusion that liver cells need less ATP to survive than other cells or that the ATP which is present is turning over much faster and providing an adequate amount of high energy phosphate to maintain viability. Thus observation of low ATP levels in cell injury systems should be interpreted with caution.

Dr Maroko

I would like to comment on two points. First, I think that simply by measuring enzymatic activities or any other substances in the heart we will not be able to define when irreversible injury occurs. By carrying out these measurements simultaneously with a histological examination it will be possible to show that necrosis occurs simultaneously with a change in an enzymatic marker but the casualty as far as the irreversibility is concerned cannot be proven. However if we hypothesize that irreversibility is dependent on a lesion to the cell membranes then the measurement of membrane ATPase as done by Drs Belle and Smith, combined with measurements of changes in permeability as shown by Dr Jennings, may offer the sought index.

The second point is of a more generic nature. It is quite conceivable that in order to decrease necrosis one does not ha o to know what irreversibility is. We do not know how to define exactly neither life nor death but we know that they are the opposites of each other. This is the definition given by the French encyclopedists and there is no better one. The biochemical and structural definitions of irreversibility will undoubtedly open new horizons however from a therapeutic point of view it is quite satisfactory to demonstrate that irreversible cell damage was prevented. This defines a successful therapy even if we do not know which event is responsible for the irreversibility that we had just prevented.

Dr Thomas

I hesitate to make a remark after that but could I ask Dr Williamson whether he thinks it is appropriate to draw distinction between an "instantaneous value" of a substance like ATP and "turnover". We attach much importance to instantaneous values I think for obvious reasons as it is easy to measure values in a biopsy. In terms of energy supply it is perhaps something rather different from that, that we need to know.

Dr Williamson

I could not agree mor and that is why some of the data I presented was in terms of ATP turnover. In many models however and certainly in humans it is extremely difficult to measure turnover. Levels of creatine-phosphate or ATP show something but not very much.

Dr Morgan

The point I was trying to make about experimental studies and thei value for clinical therapy is that since it is difficult clinically to characterize the amount of tissue that has been irreversibly damaged and to measure the exact size of an infarct, what one should hope to get from experimental studies is a rational basis for the type of therapy that is going to be introduced clinically. Perhaps that is too much to ask but before something is tried in a system as complex as man s it would be reasonable to know that it had a rational basis in some experimental system.

Dr Williamson

I think one of the problems in studying ischemia is that it is difficult to define the degree of ischemia. One should focus on the degree of oxygenation. The cell does have some good oxygen indicators namely the cytochroms and myoglobin. We are at

tempting to define the degree of ischemia more exactly from the oxygenation, disoxygenation of myoglobin in the perfused interventricular septum This is a nice flat muscle which allows one to shine light through it and to pick up redox changes that occur I think this would be an appropriate model for specifically looking at factors which might influence the ischemic intervention.

Dr Morgan

About two weeks ago there was a symposium on this general topic in Dallas and at that time Dr Chance presented some experimental data indicating that the degree of oxygenation of myoglobin went from completely oxygenated to completely deoxygenated over a very narrow area of myocardium around an infarct and presented the hypothesis that there was not such a thing as a border zone or a partially ischemic zone The suggestion was made that the cell were either oxygenated or deoxygenated and the whole concept of the border zone was a mistake I wonder if anybody would like to react to that

Dr Sobel

Perhaps we need a new word There really is no problem in reconciling those two views if one considers the border zone to be a border zone of iability rather than a border zone of oxygenation. The precision of the physical def nition of the oxygenated compared to the nonoxygenated zone or metabolically aerobic compared to the metaboli'ly anaerobic zone may not be critial The critic l question may be how long one of those zones can survive If an intervention is protective and prolongs the interval of survival then it may also modify the physical pace in which a zone of viability persi ts. This notion may also be important with regard to the i sue that was raised concerning turnover ersus steady tate concentrations. Since we are often unable to measure turnover of substances of interest we may ha e to infer that if the heart is d ing les work and maintaining a constant amount of energy store that concentration provides a reasonable index albeit indirect of turno er When contractil ty increases or decreases at the same time ATP stores are changing of course interpretation of concentrations of ATP becomes difficult. The two ideas are related since estimation of the status of a border zone of iability is related to the interval during which changes in concentration of essential components persist in relation to the work performed in that zone at the same time

D Morgan

You are arguing that Dr Williamson proposal is not going to work since it does not reflect the border zone or zone of impaired viability

Dr Jennings

I think that it is important to remember that an area of ischemia is an area of reduced arterial flow By definition it i an area where the oxygen demand exceeds the oxygen being upplied by the depressed arterial flow Moreover another factor also enters into the equation When arterial flow is reduced there is impaired diffusion of the end products of anaerobic glycolysis, uch as lactate from the ischemic ti sue The lactate accumulates both because of excess production and impaired diffusion The tissue becomes more acid What I have just described is what occurs *in vivo* after permanent coronary occlusion This picture should be remembered when assessing the various models of anoxia and ischemia *in vit o* One cannot equate anoxia with continued perfusion in a Langendorff apparatus to anoxia with low or absent perfusion. In the latter instance intermediates of glycolysis will accumulate while in the former instance the intermediates will be washed away

I also think that the baboon work demonstrated by Dr Bruyneel nicely demonstrated the zone of absent function characteristic of a large area of ischemia A similar zone can be seen in experimental myocardial infarction in dogs. There seems to me to be a rather sharp boundary between ischemic tissue i.e cyanotic acontractile tissue functioning by anaerobic metabolism and tissue which is functioning The question here is where is the border zone Is it inside or outside the zone of non-function Since the flow reduction is less marked at the periphery than in the center of the infarct I believe that the border zone if any is inside of the region of absent function, shown so clearly by Dr Bruyneel.

Dr Morgan

I think this discussion has been quite useful in clarifying some of the problems I think it is now time to adjourn this ses ion and thank the participants for their presentations

EFFECTS OF ANOXIA AND SEVERE ISCHEMIA ON THE TURNOVER OF MYOCARDIAL PROTEINS

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INTRODUCTION

Anoxia inhibited the incorporation of radioactive amino acids into proteins of heart muscle (1) and several other tissues (2 9). Although amino acid transport was sensitive to energy supply in isolated atria (4), anoxia inhibited protein synthesis in perfused hearts principally at the level of ribosome-catalyzed reactions (1). Furthermore anoxia inhibited protein breakdown in liver slices (10, 11) presumably due to an energy-requiring step in the proteolytic pathway In preliminary experiments (12), similar observations were made in isolated perfused hearts

In studies of the control of glycolysis anoxia and ischemia had different effects on the rate of myocardial glucose utilization (13 14) While anoxia accelerated the rate ischemia was inhibitory Therefore it was of interest to investigate the possibility that anoxia and ischemia had differing effects on protein turnover In the present studies established methods of ischemic perfusion (13) were modified to maintain a minimal coronary flow for 60 minutes. This period was required to estimate rates of protein synthesis and degradation. Rates of protein turnover were investigated in both anoxic and ischemic hearts and modification of these rates was attempted by provision of insulin (15) or palmitate (16)

METHODS

Perfusion of hearts

Aerobic and anoxic hearts were perfused retrograde (17) at a pressure of 60 mm Hg with Krebs-Henseleit bicarbonate buffer containing 15 mM glucose and normal plasma levels of amino acids, except phenylalanine Aerobic buffers were gassed with 95 % oxygen. 5 % CO_2 anoxic buffers with 95 % nitrogen. 5 % CO_2. After a preliminary perfusion period of 70 minutes during which the buffer passed through the heart a single time recirculation of buffer was begun and continued for the period indicated. Buffers used for recirculation were equilibrated with the appropriate gas mixture and contained ^{14}C phenylalanine amino acids and glucose as listed above and 3 % bovine serum albumin. Palmitate (1.5 mM) or insulin (25 mU/ml) was added as indicated. The first 10 ml of this buffer to pass through the heart was discarded. These hearts were not paced

Hearts were made severely ischemic by a technique similar to the "low output high aortic resistance method of Neely *et al* (13). The perfusion apparatus is diagrammed in Fig. 1 and is based on the standard working heart apparatus (18) Following aortic cannulation, a preliminary period of retrograde perfusion was begun from a reservoir at a hydrostatic pressure of 60 mm Hg by opening tube A (tubes B and C closed). The left atrial appendage was then secured to the atrial cannula. During the period of preliminary perfusion, the speed of the pump supplying perfusate to the atrial bubble trap was adjusted to 5 ml/min and the overflow was set to present a perfusion pressure of 20 mm Hg to the heart. A 50 min period of preliminary perfusion was allowed, during which rates of protein synthesis and degradation became dependent upon additions to the perfusate (12, 16) After 45 minutes electrical pacing was begun at a rate of 300 beats per minute At 50 minutes, tube A was closed tube B was opened, and recirculation of buffer was begun. Since no aortic outflow was allowed, coronary flow was equal to the speed of the pump (5 ml/min) and aortic pressure reflected the resistance of the coronary bed As shown in Fig. _, an immediate decrease in left atrial filling pressure occurred. Perfusion under these conditions resulted in decreased coronary flow and a rapid onset of

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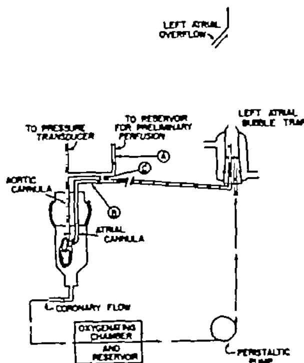


Fig. 1. Apparatus for perfusion of the severely ischemic rat heart.
The figure is described in the te t. The portions of the apparatus not diagrammed were described previously (18). Reproduced with permi sion of Circulation Research (19).

myocardial ischemia and failure as evidenced by decreased pressure development and by in creased left atrial pressure. Peak systolic pressure dropped about 70 % coronary flow de sed from 1 to ml/min. Viability of the heart a prolonged by opening tube C between the atrial and aortic cannula, two minutes after left atrial pressure had again reached 20 mm Hg. Subsequently the heart was perfused with a pres sure of 70 mm Hg supplied to both the aorta and left atrium. Twenty minutes were allowed for development of stable but reduced levels of coronary flow. Rates of protein synthesi and degradation were est mated during the f llowing one hour pe riod. Aerobic heart were paced electrically to erve a control for the ischemic preparation (300 beats per min).

Estimation of ti e levels of nucleotides and creati pho phate. For the analysis of nucleotide content, heart were f ozen during perfusion using Wollenberger tongs and powdered in a percu sion mortar at liquid nitrogen temperatures. Samples for estimation of dry weight and preparation of per chloric acid extract were handled a described earlier (16). Tissue level of creatine phosphate were determined enzymatically in perchloric acid extracts (20). Level of ATP, ADP, AMP and GTP were estimated using a Dupont liquid chromatography apparatus as described previously (4).

Analysis of intracellular amino acid content and incorporation of ¹⁴C phenylalanine into protein. Heart and perfusate amino acid content was estimated by ion exchange chromatography. Intracellular amino acid levels were calculated assuming a total water content of 809 and 841 μl/g in aerobic and in anoxic or ischemic hearts respectively. ¹⁴C Sorbitol space was assumed to be 315 μl/g in aerobic hearts and 440 μl/g in anoxic or ischemic tissues. Incorporation of ¹⁴C phenylalanine into whole heart protein was determined using a gas flow planchette counter (21). When rates of

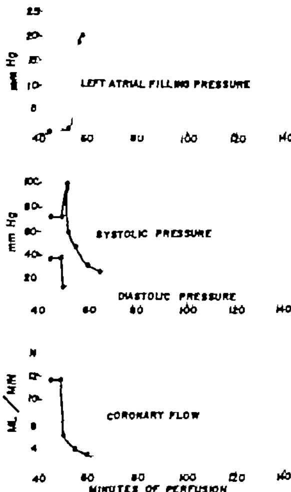


Fig. Function of the severely ischemic rat heart.
Heart were perfused described in the text. After a 45 min period of preliminary retrograde perfusion, electrical pacing w begun. After 50 min aortic inflow was topped and flow into the left atrium begun. The time course of changes show in thi figure w not affected whether the perfusate contained glucose with or without insulin or palmitate. Reproduced with permi sion of Circulation R search 19)

Table 1 Levels of nucleotides and creatine phosphate in aerobic and severely ischemic hearts.

Condition of perfusion	ATP	ADP	AMP	GTP	CrP
			μmoles/gram dry weight		
Aerobic	17±2	4 7±0.5	0.8±0._	0.6±0.1	?9±
Ischemic	8±1[a]	5 9±0.5	3 1±0.6[a]	0 15±0 02[a]	11±0 8

All hearts were paced and were perfused as described in "Methods" Ischemia was begun after 90 minutes of preliminary perfusion and nucleotide levels were determined after 100 minutes, using high-pressure liquid chromatography Creatine phosphate was determined enzymatically Data represent the mean ± standard error

a = p <0.025 vs corresponding aerobic control.

phenylalanine incorporation were estimated, perfusate phenylalanine was 0.8 mM Under these conditions specific activities of perfusate and intracellular phenylalanine were equal (23)

Estimat of the latency of lysosomal enzyme activities For studies of lysosomal enzymes, hearts were homogenized in 10 volumes of a buffer containing 0.25 M KCl 1 mM EDTA, pH 7 4 using a polytron homogenizer as detailed previously (1). Latency of enzyme activity was studied in the resulting whole homogenate ± 0._ % Triton X 100

RESULTS AND DISCUSSION

The data in Table 1 show that tissue levels of ATP and creatine phosphate were reduced in ischemic myocardium. Values in aerobic control hearts were comparable to those in unperfused tissues (16) Furthermore nucleotide analysis showed that GTP was reduced 75 % by ischemia, AMP increased 4-fold, while ADP was unchanged. Similar changes in these nucleotides were observed in anoxic hearts Insulin did not prevent these changes in high energy phosphate stores.

Incorporation of ^{14}C phenylalanine into whole heart protein was inhibited 60 % by anoxia (Fig. 3) when buffers contained 15 mM glucose amino acids, and 10 times the normal level of phenylalanine Provision of insulin or palmitate nearly doubled incorporation in unpaced aerobic hearts but had no effect in anoxic tissues These results support those reported previously which suggested that oxidation of fatty substrates was required to obtain an optimal effect on protein synthesis (16). Insulin and palmitate had similar effects in paced aerobic hearts but did not prevent the inhibition of phenylalanine incorporation observed during ischemia. Pacing of aerobic hearts at 300 beats per minute had no effect on phenylalanine incorporation.

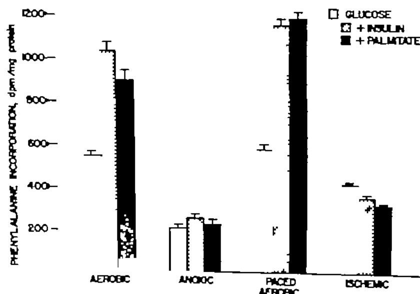


Fig 3 Effect of anoxia and ischemia on incorporation of [^{14}C] phenylalanine into whole heart protein. Hearts were perfused as described in "Methods" for total of 130 min. I unpaced aerobic and anoxic hearts, rates of phenylalanine incorporation were estimated during the final 60 min of perfusion. I paced aerobic and ischemic hearts, [^{14}C] phenylalanine was added after 65 min and rates of incorporation were estimated between 100 and 130 min of perfusion. Values in the figure represent the mean ± standard error of 8 to 42 observations.

Table 2. Effects of aerobic anoxic and ischemic perfusion on intracellular levels of amino acids.

Amino Acid	Intracellular concentration, 10^2mM			
	In vivo	Aerobic	Aerobic	Ischemic
Aspartic Acid	99±11	538±89	118±11[b]	350±45
Asparagine	22±2	68±3	79±7	80±2[b]
Glutamic Acid	826±67	503±54	409±16	477±18
Glutamine	1038±130	1078±60	1771±71	1464±93
Serine	4±3	66±3	66±4	74±8
Threonine	33±4	68±3	75±3	70±
Glycine	62±4	116±4	128±3	116±4
Alanine	110±12	165±34	660±46[a]	716±42[c]
Methionine	5.6±0.6	1[illegible].6±0.7	8.0±1.6[b]	16.9±0.4[c]
Valine	14.7±3.9	13.1±1.6	31.8±1.4[c]	25.6±0.8[c]
Leucine	11.2±1.5	14.7±1.4	30.4±1.3[c]	28.9±3.1
Isoleucine	6.8±0.9	8.[illegible]±1.2	20.7±0.8[c]	20.1±0.6
Phenylalanine	5.9±1.0	8.1±0.2	8.6±0.5	8.4±0.6
Tyrosine	8.0±2.0	9.7±0.3	9.9±0.4	8.6±0.4
Lysine	93±6	179±5	193±12	169±9
Histidine	19±	20±1	19±	17±1
Arginine	24±3	64±2	70±3	58±3
Tryptophan	–	6.6±0.5	8.1±1.0	8.1±1.2

Hearts were perfused as described in "Methods" neither insulin nor palmitate was supplied. Both anoxia and ischemia were begun following 50 minutes of perfusion. Intracellular levels of amino acids were determined after 100 minutes of perfusion. Aerobic values represent the combined data from unpaced and paced aerobic hearts, which did not differ significantly. Values shown are the mean ± standard error of 4-7 determinations using pools of 4 hearts each.

a = $p < 0.05$ vs *in vivo*

b = $p < 0.05$ vs aerobic

c = $p < 0.01$ vs aerobic

The data in Table 2 indicate that the inhibition of phenylalanine incorporation in energy-depleted hearts was not associated with reduced intracellular stores of amino acids which would serve as substrates for the synthetic pathway. After 100 minutes of aerobic perfusion, the intracellular concentrations of all amino acids except glutamate remained at or above levels observed *in vivo*. Intracellular amino acid levels were unchanged by electrical pacing of aerobic hearts and data from paced and unpaced aerobic tissues are combined in the table. Intracellular levels of aspartate and methionine decreased in anoxic hearts but remained above levels found *in vivo*. Both anoxia and ischemia increased intracellular levels of valine isoleucine and leucine perhaps by inhibition of their oxidation (22). Acceleration of glycolysis in these tissues was thought to lead to increased intracellular alanine.

In other experiments (19) decreased rates of phenylalanine incorporation in energy-poor hearts appeared to be associated with inhibition of the ribosome-associated reactions of peptide-chain initiation and elongation. Neither insulin nor palmitate restored normal ribosome cycle activity. Inhibition of these reactions in energy-poor tissues is consistent with the energy requirements of initiation and elongation reactions observed in cell free systems.

Other experiments were directed at determining the effects of anoxia and ischemia on rates of protein degradation. Hearts were perfused by the same techniques outlined for the synthesis studies. Buffers contained 15 mM glucose normal plasma levels of amino acids and 0.01 mM phenylalanine. Lowering phenylalanine to this concentration 1/8 times the normal plasma level, did not affect the rate of protein synthesis or degradation (12 23).

In the isolated perfused rat heart rates of protein degradation may be estimated by measuring the rate of dilution of the specific activity of the free phenylalanine pool and calculating the amount of non-radioactive phenylalanine which would have been released from protein to account for this dilution (1). When estimated by this method rates of protein degradation in ischemic hearts were inhibited 80 % as compared to paced aerobic tissues. When hearts were made anoxic proteolysis was reduced 60 %. Although provision of insulin inhibited proteolysis 50 % in aerobic hearts (1) the hormone had no effect in ischemic or anoxic tissues. The accuracy of estimates of rates of protein degradation made by this technique depend upon rapid mixing of phenylalanine derived from protein degradation with the total free phenylalanine. If phenylalanine from proteolysis were reincorporated into protein before complete mixing had occurred rates of proteolysis

would be underestimated. In previous experiments (17) when reincorporation of the amino acid into protein was blocked by addition of cycloheximide to the perfusate the estimated rate of protein degradation increased somewhat in aerobic hearts. However the inhibitory effects of insulin, ischemia and anoxia on the release of free phenylalanine were observed even in the presence of the inhibitor

Estimates of proteolysis by the method outlined are dependent upon release of free amino acid as the product of degradation If proteolysis were accelerated in anoxic or ischemic tissue but proceeded only to the production of peptides the acceleration would not be detected by these methods. Peptides have been reported to result from proteolysis in liver (25)

Lysosomes represent a candidate for a role in the pathway of proteolysis in heart muscle (12) In previous studies (1) we reported that during aerobic perfusion in the absence of insulin decreased latency of β-acetylglucosaminidase and cathepsin D paralleled an increase in the rate of protein degradation. At the same time development of larger autophagic vacuoles was observed by electron microscopy in the nuclear pole zone and in the rows of mitochondria (26). Addition of insulin to the perfusate of these aerobic hearts prevented increases in the rate of proteolysis and changes in lysosomal latency Autophagic vacuoles did not increase in the presence of the hormone These observations led us to examine changes in lysosomal latency in energy-poor hearts.

Severe ischemia reduced the latency of myocardial β-acetylglucosaminidase activity somewhat but was without effect on the latency of cathepsin D In these experiments latent enzyme was considered to be increment of activity which was assayable only upon addition of Triton X 100 to the whole homogenate. Although provision of insulin to paced aerobic control hearts increased latency of both enzymes, the hormone had no effect in ischemic tissues.

In similar experiments, anoxia reduced the latency of both β-acetylglucosaminidase and cathepsin D following one hour of perfusion. Insulin maintained increased latency in unpaced aerobic tissues, but had no effect in anoxic hearts Decreased latent enzyme activity was thought to reflect increased fragility of lysosomes during the homogenization procedure. Larger lysosomes may be susceptible to mechanical disruption during homogenization Their presence may account for decreased latency

Thus, when heart were perfused by a method designed to produce severe ischemia, rates of phenylalanine incorporation were inhibited. Reduced rates of incorporation did not appear to involve depletion of amino acids from ischemic tissues, but resulted from inhibition of the energy-requiring reactions of peptide-chain initiation and elongation. In contrast to aerobic hearts, provision of insulin or fatty acid during ischemia did not prevent the inhibition of protein synthesis. Similar results were obtained in anoxic hearts. Rates of protein degradation increased and latency of lysosomal enzyme activity decreased when hearts were perfused under aerobic conditions. These changes were not affected when hearts were paced electrically at 300 beats per minute When insulin was provided changes in proteolysis and latency were prevented. In hearts perfused under anoxic or ischemic conditions protein degradation was inhibited, but latency of lysosomal enzymes decreased. Inhibition of protein degradation in association with depletion of high energy phosphate compounds, in both anoxic and ischemic hearts was consistent with an energy requirement for myocardial proteolysis.

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DISCUSSION

Dr Hjalmarson

Thank you Dr Morgan for your interesting presentation The paper is now open for discussion

Dr Sobel

Just a brief question Does fatty acid substitute for insulin on the degradative side of the equation the way it does on the synthetic side or not

Dr Morgan

We have not investigated that in detail but if you add fatty acid during the first hour of perfusion of an aerobic heart it will prevent the increase in the rate of degradation that occurs when insulin or fatty acid is absent

Dr Braunwald

In qualifying your conclusion about the relevance of these interesting observation to patient would you comment on the possibility that ischemia might be tolerated for longer period if it were not complete Perhaps the processes which you described will not begin in 120 minutes but will take 180 minutes

Dr Morgan

I agree with you completely In a less severely ischemic model the heart will continue to beat with a 50 per cent reduction in coronary flow for hours This period is about the limit for this preparation If there are protective effects of agents that operate over a much longer time span we will not be able to study them with the isolated rat heart

Dr Hjalmarson

I am interested in your statement that the inhibition or the decrease in protein synthesis is probably due to the energy deficiency in the ischemic heart Do you have any direct evidence for this because the energy requirements for protein synthesis are very low compared to the high energy levels even in the ischemic muscle

Dr Morgan

That is right The evidence for this is that if you measure the levels of ribosomal sub-units you find that the sub-unit level will fall in an ischemic or anoxic heart The changes are not big If insulin is added peptide chain initiation is stimulated and ribosomal sub-units are low In this situation elongation of peptide chains accounts for the inhibition If the GTP is assumed to be equally distributed throughout the heart water the intracellular concentration would be 0 07 mM in ischemic cell This is 5 10 times higher than the Km of the elongation enzymes I think there are a number of unsolved problems regarding compartmentation of GTP and the regulation of the elongation enzymes These enzymes appear to have a higher Km for GTP in the intact heart than they do in the test tube Additional studies are needed to resolve the control of activity of these enzymes in the intact cell I agree there is a problem.

Dr Hjalmarson

You showed one slide where you paced hearts at 300 beats/min under aerobic conditions and especially with palmitate you got an increase in the rate of protein synthesis You said that if you pace at 400 beats/min you get even a more marked increase in protein synthesis What about protein degradation under those conditions and what do you think about the mechanism Why will pacing in itself increase rate of protein synthesis

Dr Morgan

We have not looked at higher pacing rates on protein degradation as yet. A rate of 300/min as compared to 220 to 240 does not have any noticeable effect. In terms of the mechanism of the effect of pacing on synthesis it may be that the rate is related to the total tension developed. It may be possible to increase total tension development more by increasing rate than by increasing outflow resistance or atrial filling pressure I mean it is difficult to maintain a peak systolic pressure above 140 and 150 mm Hg in isolated hearts whereas you can double the rate by pacing. It may be that rate is as important as the peak systolic pressure development in leading to cardiac hypertrophy

Dr Hjalmarson

We have performed studies showing that the heart can be arrested with lidocaine or beta-blockers and nothing will happen with the rate of protein synthesis. Quinidine or procainamide at high concentrations will arrest the heart and reduce the rate of protein synthesis

Dr Morgan

We did some of this type of experiment too by stopping the heart with tetrodotoxin. These hearts had the same rate of synthesis as those that were beating 220 beats/min. On the other hand, this observation does not rule out the possibility that increasing heart rate above 220 beats/min will accelerate synthesis

Dr Elsasch

What is the magnitude of this protein synthesis and breakdown Does it have any significance on the body total protein turnover like for instance the kidneys have been postulated to have

Dr Morgan

I do not think the heart *per* is going to contribute very much to the total amino acid metabolism of man. It is important to realize how rapidly heart protein turnover is and that if synthesis stopped and degradation continued some enzymes could virtually disappear in 6-8 hours

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I am interested in your statement that the inhibition or the decrease in protein synthesis is probably due to the energy deficiency in the ischemic heart Do you have any direct evidence for this because the energy requirements for protein synthesis are very low compared to the high energy levels even in the ischemic muscle

Dr Morgan

That is right The evidence for this is that if you measure the levels of ribosomal sub-units you find that the sub-unit levels will fall in an ischemic or anoxic heart The changes are not big. If insulin is added peptide chain initiation is stimulated and ribosomal sub-units are low In this situation elongation of peptide chains accounts for the inhibition If the GTP is assumed to be equally distributed throughout the heart water the intracellular concentration would be 0 07 mM in ischemic cells. This is 5-10 times higher than the Km of the elongation enzymes I think there are a number of unsolved problems regarding compartmentation of GTP and the regulation of the elongation enzymes. These enzymes appear to have a higher Km for GTP in the intact heart than they do in the test tube Additional studies are needed to resolve the control of activity of these enzymes in the intact cell I agree there is a problem.

Dr Hjalmarson

You showed one slide where you paced hearts at 300 beats/min under aerobic conditions and especially with palmitate you got an increase in the rate of protein synthesis. You said that if you pace at 400 beats/min you get even a more marked increase in protein synthesis. What about protein degradation under those conditions, and what do you think about the mechanism Why will pacing in itself increase rate of protein synthesis

EFFECTS OF METABOLIC AND PHARMACOLOGIC INTERVENTIONS ON MYOCARDIAL INFARCT SIZE FOLLOWING CORONARY OCCLUSION

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ABSTRACT

A number of hemodynamic, pharmacologic and metabolic interventions were found to change the extent of acute ischemic injury of the myocardium and subsequent necrosis following experimental coronary artery occlusion. Reduction in myocardial damage occurred by decreasing myocardial oxygen demands (beta-adrenergic blocking agents, intra-aortic balloon counterpulsation, external counterpulsation, nitroglycerin, decreasing afterload in hypertensive patients, inhibition of lipolysis, and digitalis in the failing heart) by increasing myocardial oxygen supply either directly (coronary artery reperfusion or elevating arterial pO_2) or through collateral vessels (elevation of coronary perfusion pressure by alpha-adrenergic agonists, intra-aortic balloon counterpulsation) or by increasing plasma osmolality (mannitol, hypertonic glucose), presumably by augmenting anaerobic metabolism (glucose-insulin-potassium, hypertonic glucose), by enhancing transport to the ischemic zone of substrates utilized in energy production (hyaluronidase), by protecting against autolytic and heterolytic damage (hydrocortisone, cobra venom factor, aprotinin). Augmentation of myocardial ischemic damage occurred as a consequence of increasing myocardial oxygen requirements (isoproterenol, glucagon, ouabain, bretylium tosylate, tachycardia), by decreasing myocardial oxygen supply either directly (hypoxia, anemia) or through reduction of collateral flow (hemorrhagic hypotension, minoxidil) or by decreasing substrate availability (hypoglycemia). Pilot studies have been carried out in patients with hyaluronidase, nitroglycerin, intra-aortic balloon counterpulsation, beta-blocking agents and Arfonad and have shown that these interventions may also reduce myocardial damage, suggesting that the concept of reduction in infarct size following coronary occlusion is applicable clinically.

Key Words

Myocardial oxygen consumption, beta-adrenergic blocking agents, intra-aortic balloon counterpulsation, digitalis, coronary artery reperfusion, hyaluronidase, myocardial infarction, electrocardiographic ST segments, electrocardiographic QRS complex

It has been suggested that any therapeutic modality that could decrease the extent of myocardial necrosis after coronary occlusion and, in this manner, reduce the frequency and severity of pump failure would be expected to be extremely useful not only for lowering the immediate mortality of acute myocardial infarction, but perhaps even more importantly, might leave patients who had suffered coronary occlusions with a greater quantity of viable myocardium (1-3). Such patients would have a greater reserve of functioning myocardium should another coronary occlusion occur at a later time and would also be expected to be less likely to develop chronic heart failure.

In order to test this hypothesis and to evaluate the possible applicability of this therapeutic approach to patients, our investigations were carried out in six stages: 1. Determination of whether the extent of necrosis is determined principally by the site of occlusion and anatomic factors such as the degree and location of collaterals or whether infarct size can be altered following coronary artery occlusion. 2. Identification of the physiolo-

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tion despite a several-fold increase in blood delivery to the nonischemic myocardium the regional blood flow both to the periphery and to the center of the ischemic zone declined (19) This effect caused an increase in ischemic injury showing the detrimental effect of this type of intervention (16) On the other hand, nitroglycerin has been found to redistribute the flow in the ischemic area in a more favorable fashion (20) and to reduce myocardial damage presumably by acting through this mechanism (14 15) This effect of nitroglycerin is augmented when it is administered in combination with methoxamine which returns the arterial pressure to its original level All of these observations when taken together strengthen the hypothesis that the extent of myocardial ischemic injury following coronary occlusion is influenced importantly by the balance between myocardial oxygen supply and demand.

These studies suggest that conditions such as tachycardia and hypotension or the combination occurring in a patient with an acute coronary occlusion might be detrimental extending the size of the ischemic zone and thereby further impairing left ventricular function thereby lowering arterial pressure further and resulting in a vicious cycle They also show that the administration of positive inotropic agents especially in the absence of heart failure may be detrimental in a similar manner and they point to the potential hazards of certain antiarrhythmic agents such as bretylium tosylate and of some coronary vasodilators which may result in an unfavorable distribution of flow in the myocardium causing a steal phenomenon and subsequently increasing myocardial ischemic damage

The analysis of the relationship between myocardial energy supply and demand was then extended to include a consideration of anaerobic metabolism. Normally the heart derives essentially all of its energy from the oxidation of various substrates in the Krebs cycle however the myocardium does possess the capacity to derive significant quantities of energy from anaerobic glycolysis in the absence of oxygen It was reasoned that since the size of myocardial infarction is dependent on the balance between the availability and demand for various components involved in energy production then the anatomic integrity and function of cardiac muscle might be preserved by increasing energy supply through anaerobic glycolysis Accordingly the effect of the infusion of glucose-insulin-potassium and of simple hypertonic glucose was examined (4) It was found that when administered 30 minutes after coronary artery occlusion both interventions substantially decreased the quantity of necrosis as reflected in the myocardial CPK activity and histological appearance 4 hours later (Fig. 3 4). These interventions may also exert beneficial action through their hyperosmolar effects, similar to those de-

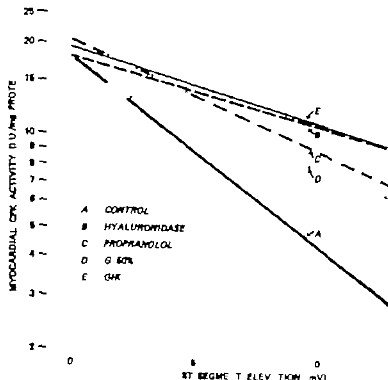


Fig. 3 Relationship between ST segment elevation 15 minutes after occlusion and log creatine phosphokinase (CPK) activity from the same specimens obtained 4 hours later Line A control group (occlusion alone). Fifteen dogs, 101 biopsies Line B hyaluronidase Thirteen dogs, 94 biopsies. Line C. propranolol. Line D glucose 50 % Six dogs, 46 biopsies. Line E. glucose-insulin-potassium infusion Thirteen dogs, 96 biopsies. All interventions started 30 minutes following coronary artery occlusion i.e. 15 minutes after the epicardial mapping. There is a statistical difference ($p < 0.01$) between the slope of line A and the slopes of the other lines showing less creatine phosphokinase depression after treatment

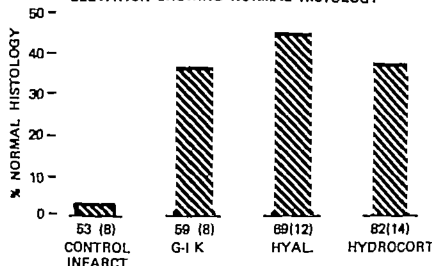


Fig. 4 Comparison of the effect of treatment on histology in areas with ST segment elevations over mV First column control group Second column glucose-insulin-potassium (GIK) group Third column hyaluronidase group Fourth column: hydrocortisone group Note that in all three treatment groups more than one third of sites that were expected to show early signs of myocardial infarction were spared Reproduced with permission of Ann. Intern Med. (7).

scribed for mannitol (21). Also the reduction in circulating fatty acids the restoration of intracellular potassium and the stabilization of cell membranes may in part have been responsible for this favorable action. In contrast hypoglycemia was shown to be detrimental increasing myocardial damage (22).

Since hyaluronidase increases diffusion through the extracellular space and may thereby facilitate delivery of substrates to ischemic cells its influence on the size of experimentally produced infarcts was also analyzed (5). This enzyme was found to decrease myocardial ischemic injury and ultimately myocardial necrosis substantially (Fig. 3 4) It was effective either as pretreatment, or when administered one-half hour following coronary occlusion. It produced a dramatic reduction in ST segment elevation and a sparing effect on myocardial CPK activity: numerous myocardial specimens, which were expected to progress to exhibit necrosis were normal 24 hours after occlusion.

Effects of interventions that limit the inflammatory reaction following myocardial ischemic cell damage Immediately following a coronary artery occlusion, a series of biochemical changes take place which culminate in anatomically recognizable necrosis The cells to which the flow is maximally limited i e those which are in the center of the ischemic zone show definite signs of irreversible injury as early as 20 minutes following occlusion (23). This process of irreversible injury evolves for many hours and as long as 18 hours following occlusion, changes in the extent of necrosis can be recognized (24) Presumably this process of defining the boundaries of a necrotic zone results not only from ischemia *per se* but from many other influences that may result either in definite irreversible damage or the sparing of these cells in the border zone Following the initial damage caused directly by the anoxic stimulus many factors that are responsible for the increase in capillary permeability interstitial edema, leukotaxis phagocytosis and nonspecific injury to cell membranes are activated or released from several enzymatic complexes. Accordingly the influence of interventions that can limit these reactions of the organism has been examined The activation of the complement system via its alternate pathway which may occur in this condition releases leukotactic factors and may be responsible for increases in capillary permeability and interstitial edema, and it may contribute substantially to the nonspecific injury to the cellular membranes (25). Accordingly the action of cobra venom factor (CVF) a protein that enzymatically cleaves C3 and thus prevents the effects of the complement system on the size of an experimentally induced myocardial infarction, has been investigated (26). Also the effects on infarct size of aprotinin an inhibitor of the kallikrein system, have been studied (27), since the kallikrein system may similarly enhance leukotactic activity capillary permeability interstitial edema and proteolytic activity Moreover the effect of glucocorticoids was examined using hydrocortisone (28) in pharmacologic doses. In addition to its action on the leukocytes hydrocortisone may also stabilize lysosomal

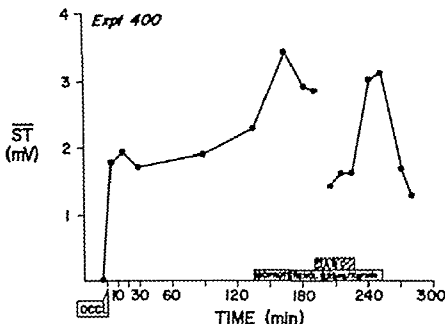


Fig. 5 An example of the influence of isoproterenol and IABC on $\overline{ST}$ Note the increase in $\overline{ST}$ following isoproterenol infusion the decrease with the beginning of IABC and its increase when IABC was discontinued. Reproduced with permission of Circulation (17).

and other cellular membranes All of these interventions were shown to be beneficial limiting substantially the extent of myocardial ischemic injury following experimental coronary artery occlusion It may be postulated that by limiting these responses of the organism to the initial injury additional damage to myocardial cells is avoided and thus the cells which are still only reversibly damaged may recover since the development of the collateral circulation occurs relatively soon following the ischemic stimulus provided by coronary occlusion

Effect of delayed interventions The analysis of all of the aforementioned esults demonstrates that several interventions can reduce infarct size when applied prior to or shortly after coronary artery occlusion This concept however will be of l mited clinical application if these interventions are not effective several hours after the onset of the coronary obstruction i e at a time when most patients have reached the hospital Accordingly many of these interventions were examined when administered up to six hours after coronary artery occlusion (Fig. 5) (3 4 7 17 28-31) It was found that isoproterenol propranolol methoxamine neosynephrine hydrocortisone hemorrhagic hypotension intra-aortic balloon counterpulsation, and the combination of glucose-insulin-potassium and propranolol and norepinephrine can reduce the extent and magnitude of myocardial schemic injury when administered as late as 3 to 6 hours after coronary occlusion, in a manner similar to that observed when they were administered before or immediately after the occlusion

Ext apolation of the experimental results to the clinical situation The principal conclusion resulting from all of these studies is that the myocardial tissue in the zone of distribution of a vessel which has become occluded is not necessarily destined to become irreversibly damaged. Rather following coronary artery occlusion substantial portions of the myocardium remain reversibly injured for a number of hours and may progress either to necrosis or to complete recovery The reduction of myocardial oxygen needs the improvement of coronary perfusion the provision of energy by means of anaerobic glycolysis the improvement of opportunity of dif fusion of oxygen and/or substrates to ischemic cells and the possible reduction of the autolytic and heterolytic processes precipitated by the initial ischemic event all appear to be capable of favorably altering the state of the myocardial tissue mostly at the border of the ischemic myocardium, where a larger percentage of the cells are still reversibly damaged than at the center Of equal importance is the increase in infarct size following coronary occlusion which might occur as a consequence of the deleterious effects of certain hemodynamic and metabolic conditions and as a consequence of the administration of several drugs.

Since the experimental models never can mimic perfectly the clinical setting it was imperative to develop techniques that would be able to measure changes in infarct size atraumatically in patients with acute myocardial infarction For this purpose, a method using precordial electrodes was developed that could evaluate changes in myocardial inju-

ry (31). It was observed that, using multiple precordial electrodes in the dog, the changes in myocardial ischemic injury as measured by ST segment elevations parallel those observed on the epicardium, the latter of course had been shown capable of predicting necrosis by both histologic and enzymatic criteria (31, 32). Using precordial leads in dogs it was shown that isoproterenol and hemorrhagic hypotension increase myocardial ischemic injury while propranolol, hypertension and reperfusion reduce it.

Clinical observations. Utilizing a system similar to that used in the closed chest dog, multiple precordial leads were utilized to measure electrocardiographic changes in man (31). The system consisted of 35 precordial electrodes, connected to a clinical electrocardiograph. This method permitted the study of changes in ST segment elevation in patients with anterior, antero-lateral and high lateral transmural acute myocardial infarctions.

The precordial electrocardiographic technique showed that a predictable reduction in the magnitude of ST segment elevation occurs as a function of time in patients with uncomplicated infarcts and suggests that factors that affect the magnitude of ischemic injury observed in the dog with acute coronary occlusion are also operative in man. It was observed in individual patients that an increase in ST segment elevation occurs as a consequence of arterial hypotension (31), recurrence of ischemic pain (31), ventricular fibrillation (31) and that a reduction occurs after propranolol administration (31), and during intra-aortic balloon counterpulsation (17). These initial studies were followed by others that showed that propranolol (33) and intra-aortic balloon counterpulsation (34) reduce ST segment elevation in patients with acute myocardial infarction and improve the metabolism of the ischemic heart as reflected by lactate extraction (35, 36). Also, recent investigations have shown that hyaluronidase (37-39) and nitroglycerin (40-42) limit infarct size.

One may speculate that in the future two types of interventions will be applied to reduce infarct size in patients. One type will include those which might be applicable in every patient in the early stage of infarction, such as those that act on the inhibition of harmful reactions of the organism, i.e. aprotinin, cobra venom factor and glucocorticoids, hyaluronidase, which may facilitate the transport of nutrients to the ischemic zone and/or the washout of noxious substances from that same zone might also be included in that group. The second group of intervention will be those useful in a specific hemodynamic or metabolic situation. Their administration will de-

Table 1. Interventions that modify myocardial injury following coronary occlusion.

I. Interventions that reduce myocardial injury
- A. by decreasing myocardial oxygen demand
  - 1. propranolol*
  - 2. practolol*
  - 3. cardiac glycoside in the failing heart
  - 4. counterpulsation
    - a. intra-aortic balloon
    - b. external counterpulsation
  - 5. nitroglycerin
  - 6. by decreasing afterload in hypertensive individuals – Arfonad
  - 7. by inhibition of lipolysis – beta-pyridyl-carbinol
- B. by increasing myocardial oxygen supply
  - 1. directly
    - a. coronary artery reperfusion
    - b. elevating arterial pO_2
    - c. thrombolytic agents
  - 2. through collateral vessels
    - a. elevation of coronary perfusion pressure by methoxamine, neosynephrine, or norepinephrine
    - b. intra-aortic balloon counterpulsation
    - c. external counterpulsation
  - 3. by increasing plasma osmolality
    - a. mannitol
    - b. hypertonic glucose
- C. by augmenting anaerobic metabolism (presumed)
  - 1. glucose-insulin-potassium
  - 2. hypertonic glucose
- D. by enhancing transport to the ischemic zone of substrate utilized in energy production (presumed) – hyaluronidase
- E. by protecting against autolytic and heterolytic processes (presumed)
  - 1. hydrocortisone
  - 2. cobra venom factor

II. Interventions that increase myocardial injury
- A. by increasing myocardial oxygen requirements
  - 1. isoproterenol
  - 2. glucagon
  - 3. ouabain
  - 4. bretylium tosylate
  - 5. tachycardia
- B. by decreasing myocardial oxygen supply
  - 1. directly
    - a. hypoxemia
    - b. anemia
  - 2. through collateral vessels – reducing coronary perfusion pressure (hemorrhage)
- C. by decreasing substrate availability – hypoglycemia

* denote interventions which have received some clinical application.

pend on the categorization of patients according to the existence or absence of heart failure the level of arterial pressure and the richness of the collateral vessels between the unoccluded and occluded coronary arteries Thus in regard to the effect of changes in arterial pressure on myocardial injury it is likely that each patient has an optimum level of perfusion pressure As coronary perfusion (aortic diastolic) pressure is elevated towards this level the perfusion of and therefore oxygen delivery to the peri-infarction zone increases more than do the oxygen needs of this tissue The ensuing more favorable balance between oxygen supply and demand will reduce the extent of ischemic injury As coronary perfusion pressure is elevated above this optimum level the increasing oxygen delivery does not keep pace with the increase in oxygen needs and ischemic injury increases. In the normal dog, this optimum pressure level is relatively high and was not exceeded in the previously described studies In patients with acute myocardial infarction this optimum level appears to be much lower and probably depends mostly on the presence or absence of collateral vessels Thus in a patient with a highly developed collateral circulation this level might be higher than in a patient with poorly developed collaterals between the normal and ischemic zones since in the latter each increment in arterial pressure will only increase the afterload without increasing further the collateral blood flow to the ischemic zone Moreover the presence of heart failure may complicate further the influence of changes in aortic pressure When the heart is already dilated as a consequence of the operation of LaPlace s law the myocardial tension and therefore oxygen consumption is elevated a reduction of arterial pressure (through its effect on ventricular afterload) could favorably alte the balance between myocardial oxygen supply and demand thereby reducing ischemic injury following coronary occlusion

In conclusion the present experimental findings which show that infarct size can be reduced following coronary artery occlusion have been applied to patients with acute myocardial infarctions with encouraging results (Table 1)

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DISCUSSION

Dr Morgan

Dr Maroko s very interesting paper is open for discussion

Dr Kjekshus

Most vasodilators abolish the auto-regulatory power of the coronary vessels and they become a passive circulatory system Flow therefore becomes dependent on the extravascular tissue pressure and will be redistributed according to the tissue pressure i.e away from the ischemic area. Do you find that other vasodilators have the same effect on the ischemic injury

Dr Maroko

I do believe that several other vasodilators should have the same effect However since I do not have ta to prove it, I elected to offer a caveat In a different category is nitroglycerin which has unique properties and its actions on the coronary arteries may be exerted at different segments of the artery than the other vasodilators Indeed nitroglycerin apparently has the beneficial effect on myocardial injury as shown by Drs Hirshfeld Kent Borer Goldstein and Epstein. Therefore I agree with your explanation but I still think that there may be a substantial difference between the effect of drugs that act on the conductance or on the resistance part of the vessels

Dr Hjalmarson

I congratulate Dr Braunwald group to your very interesting studies Howeve I have some problems while reading your papers I would like to know your experimental conditions Do you ha e any indirect measurements of sympathetic activity like plasma catecholamines f ee fatty acids or heart rate When pacing a heart catecholamines will be released and you might have a positive staircase phenomenon So it is not simply an increase in heart rate Will digitalis in a failing heart reduce heart rate more than in a non-failing heart? You can reduce the heart work by lowering heart rate and that might be more important than the inotropic effect of digitalis A slide with practolol was shown and I wonder if the basic sympathetic activity is working on that heart when you give practolol or if isoproterenol was first infused I believe that in the bottom of all the control dogs there is an elevated sympathetic activity What about the heart rate in the dogs made hypertensive? The increase in blood pressure could reflexively reduce sympathetic activity and heart work In the experiments with hypoxia the sympathetic activity will be increased which is also the case under hypoglycemia. It is obvious that measurements of sympathetic activity would be of great interest. It could be of value to repeat your experiments under very intensive beta-blockade to find out the importance of all factors isolated. Is it possible that the only important factors are sympathetic activity myocardial contractility and heart rate?

Dr Maroko

No In the series of experiments in which hypoglycemia was studied some animals were beta-blocked with propranolol and there still was a marked increase in ischemic injury following hypoglycemia. In the case of lowering the arterial pressure these experiments were carried out under barbiturate anesthesia. Hemorrhage under barbiturate anesthesia does not increase heart rate as occurs in the conscious animal or in men. Therefore the above described hemorrhagic hypotension caused increase in myoca dial injury that cannot be attributed to an increase in heart rate since the latter did not occur In the series of experiments in which myocardial injury was reduced by beta-blockade with propranolol some of the dogs were paced These paced animals also showed reduction in schemic njury In conclusion, it is clear f om ou studies that an increase of heart rate will enlarge the myocardial njury It was also demonstrated that changes in arterial pressure altered myocardial injury without altering heart rate and that propranolol alters njury with a fixed heart rate Also it was demonstrated that the interventions such as hypoglycemia will increase heart rate even in the presence of beta-blockade

In the background of you question however is apparently a fundamental difference in the approach to the question of minimizing infarct size Our main concern was to find out if thera-

peutic interventions can change myocardial injury and if so which interventions increase and which ones decrease it. Once this question, *which in our opinion is the essential one* is answered, then comes the time to define more precisely the mechanisms of action.

Dr Hjalmarson

I agree with you and I am quite sure that all the factors studied will in some way change the degree of myocardial ischemia. But I think it must be stressed that you have studied a number of factors combined with an elevated endogenous sympathetic activity. *This is important when comparing studies on* dog heart *in situ* and the isolated perfused hearts that we are working on. The isolated rat heart might be completely different because it is not under the endogenous sympathetic influence.

Dr M eller

I would like to come back once more to the use of vasodilators since they became an important therapeutic intervention in the failing heart following *acute myocardial infarction*. Their action seems different. Your observations with monoxidil indicating significant coronary vasodilations suggest that coronary autoregulation is outweighed the coronary vascular bed becomes unresponsive and dependent upon perfusion pressure. Nitroprusside in contrast, appears to be a less potent coronary vasodilator. Ganz *et al* reported a decrease of coronary blood flow in the normal myocardium, associated with decrease in cardiac work.

Dr Maroko

The only experimental study in which the effect of external counterpulsation on myocardial injury was studied is that of Johansen, DeLaria, and Bernstein in which they showed a reduction in ST-segment elevation, however no mechanism of action was studied. In the cooperative study in patients with acute myocardial infarction the mortality and morbidity rate was decreased, but again there are no data about the mechanism of action. It is probable however that the intervention *is effective through a* reduction in heart work or through an increase in collateral flow.

Dr Mjo

This w s a ery nice presentation and I just wanted to ask few questions on hyaluronidase. Is there any hemodynamic changes with hyaluronidase in your preparation and the next question is as far as I can see from your slides on the histology the normal histology in the ischemic area seems to be better *maintained* with *hyaluronidase than with* glucose-insulin-potassium or hydrocortisone. Would that mean that hyaluronidase is the drug of choice if you want to extend your data into human treatment of acute myocardial infarction.

Dr Maroko

Experimentally in dogs with or without occlusion of a coronary artery hyaluronidase did not change *arterial pressure heart rate LV dp/dt LVEDP* etc. So there was no hemodynamic effect. In hearts which were autoperfused with a Gregg cannula and in which coronary flow was reduced by steps hyaluronidase delayed heart failure showing therefore a beneficial hemodynamic effect in an ischemic failing heart.

As far as the comparison between histological results, I do not think that we can look at it in a quantitative manner because the numerical data show changes in number of biopsies with necrosis *and since the choice of sites for biopsies is arbitrary*. I believe that the results show only directional changes.

The reason why hyaluronidase was chosen by us for the first trial in patients is that it compared very favorably with the other interventions in dogs and that it was devoid of side-effects in patients when it was used rather extensively in the fifties.

Dr Braunwald

I would like to make an additional comment concerning Dr Hjalmarson s remarks. I think that there are at least two basic ways in which one can go about investigating a problem such as the one that is the subject of this conference i.e. *'Protection of the* Ischemic Myocardium'. I think that they both have some merits and that they both have some limitations. One is to carry out studies to determine whether or not a specific phenomenon exists (in this instance the phenomenon is the protection of ischemic myocardium) and then if the finding turns out to be interesting and potentially important, either try yourself or try to interest other people into explaining it. This is what Dr Maroko and my other colleagues and I have been about. We consider this to be analogous to *flying at about 35 000 feet* and taking a broad survey of the terrain seeing where the rivers lakes and mountains are and then determining which areas should and which should not be

returned to for more careful examination. I recognize that many of our observations are descriptive and do not explain the underlying phenomenon, but first it is essential to determine whether or not there is even a phenomenon that is worth explaining. Once that point has been established it then behooves a scientist to attempt to explain it. The other approach is to do a detailed analysis of a restricted issue, such as the specific mechanism of a proposed intervention, in which all of the known variables are carefully controlled. This would be akin to the detailed exploration on foot of a small portion of the land. This is obviously a useful approach, but it is also extremely risky in that one does not know whether the entire journey is worth undertaking. I think that these two general approaches should be used judiciously and complement each other. Both have a contribution to make.

EFFECT OF SUBSTRATE ON ENZYME RELEASE AND ELECTRON MICROSCOPIC APPEARANCES AFTER CORONARY ARTERY LIGATION IN ISOLATED RAT HEART*

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INTRODUCTION

It has been suggested (9 10) that the outcome of myocardial ischaemia is dependent on the nature of the substrate reaching the ischaemic zone We tested the hypothesis that provision of glucose might be beneficial and provision of free fatty acids (FFA) harmful for the survival of ischaemic infarcting tissue by perfusing isolated working rat hearts after coronary artery ligation by media containing glucose or glucose and albumin, or a long chain fatty acid and albumin in different FFA albumin molar ratios, or albumin-bound fatty acid together with glucose and insulin

METHODS

Male rats of the Sprague-Dawley strain, fed *ad libitum* were used in these experiments.

After the animal had been anaesthetized by ether and heparinized (100 IU injected into the femoral vein) the heart was rapidly excised according to the technique of Neely *et al* (7) arrested by plunging into ice-cold perfusion fluid and mounted by aorta on cannula and perfused in a retrograde manner by the Langendorff technique (6) in a non-recirculating fashion at 65 cm H_2O pressure Then the left atrium was cannulated to allow atrial perfusion ("working heart" technique) of Neely *et al* (7) as modified by Opie *et al* (8)

During the working heart period, preparations were perfused via the left atrium at an atrial pressure of 10 cm H_2O

In non-ligated hearts (control experiments) perfused with glucose as substrate the left ventricle spontaneously ejected 40 to 50 ml of perfusate per min against a hydrostatic pressure of 100 cm of water The aortic output and coronary flow were pooled and recirculated

The left coronary artery was stitched with silicone-treated silk 5-0 by means of a 3/8 circle taper needle (Davis & Jeck, Pearl River New York). The ligature was placed about 1 mm below the root of the aorta at the left bottom edge of the left atrium, as described by Bajusz (4) and by Kannengiesser *et al* (5). Substrates used included. glucose (11 mM) glucose (11 mM) and albumin (0.45 mM), palmitate (0.5 – 1.5 mM) bound to albumin (0.1 – 0 45 mM) linoleate (0.5 mM) bound to albumin (0 1 mM) oleate (0.5 mM) bound to albumin (0.1 mM), and palmitate-albumin (0.5 mM 0.1 mM) together with glucose (11 mM) and insulin (2 mU/ml)

Release of enzymes is thought to be related to loss of integrity of the cell membrane Therefore to check the extent of cellular alterations we measured lactate dehydrogenase (LDH) activity in perfusate by the method of Wroblewski *et al* (11). Methods for electron microscopic examination are fully described elsewhere (?)

RESULTS

Cardiac output after coronary artery ligation was lowest with palmitate (28-38 % of initial value) and highest with glucose (61 %). LDH release was 5-10 x greater with palmitate-albumin as substrate than with glucose or glucose-albumin. Increasing the molar ratio of palmitate to albumin from 1 1 to 5 1 doubled the rate of release of LDH The addition of

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* Full texts submitted for publication elsewhere (1-3).

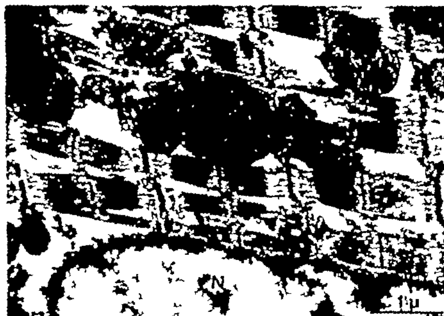


Fig. 1 Effect of coronary artery ligation on ventricular ultrastructure of isolated working rat heart perfused with palmitate bound to albumin (molar ratio 3) as substrate. This longitudinal section shows the appearance of ischemic myocardium with peripheral *aggregation of the nuclear* chromatin distortion of the nuclear membrane relaxation of the myofibers and prominent interfibrillar spaces absence of glycogen. Note the electron dense bodies (arrow) inside of the mitochondria. *M* mitochondria *N* nucleus

glucose and insulin to palmitate-albumin decreased release of *LDH* by two-thirds.

In ligated heart perfused with palmitate albumin mitochondria appeared very damaged with electron-dense bodies possibly *representing* unmetabolized lipids or calcium deposits as suggested by Jennings *et al* (1) Such intramitochondrial bodies were never found in glucose-perfused hearts When glucose and insulin were added to palmitate albumin-perfused hearts the incidence of dense bodies was considerably less

CONCLUSION

We conclude that the extent of myocardial cell damage resulting from coronary ligation assessed by release of enzyme and by electron microscopic changes is greatly increased by the presence in perfusing fluid of FFA and decreased by the additional presence of glucose and insulin. Our results argue for an important role for substrate effects on the metabolic outcome of experimental myocardial infarction

Table 1 Effect of various substrates and of insulin on the rate of release of lactate dehydrogenase (LDH) from isolated perfused working rat heart with coronary artery ligation.

Substrates and insulin concentration	Albumin concentration	FFA/Albumin molar ratios	LDH lease $U \times g \times h^{-1}$
glucose 11 mM	0.44 mM	–	2.6±0 15 (5)
palmitate 0 5 mM	0 44 mM	1 1	10 7±1 17 (8)
palmitate 1 5 mM	0 44 mM	3 4	19 5±2.51 (4)
palmitate 0 5 mM	0 10 mM	5.0	23 3±1 77 (8)
palmitate 0 5 mM + glucose 11 mM	0 10 mM	5 0	15 3±0.57 (6)
palmitate 0 5 mM + insulin 2 mU/ml	0 10 mM	5 0	12 9±2.44 (5)
palmitate 0.5 mM + glucose 11 mM + insulin mU/ml	0 10 mM	5.0	8 1±1 11 (6)

Mean values S E M (number of hearts)

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DISCUSSION

Dr Morgan

In hearts with only fatty acid in the buffer there would not be any substrate for the oxygen deficient cell. One would anticipate very rapid depletion of ATP and cell death as compared to a cell that was provided a substrate for anaerobic glycolysis. I realize you showed a further decrease in enzyme release when insulin was added even though both fatty acid and glucose were present. I was worried about comparing fatty acid alone to glucose alone.

Dr Fitzgerald

I realize that cardiac arrhythmias are difficult to produce in the isolated rat heart preparation, but did you by chance observe any change in the electrical activity in the different groups of rat hearts that you studied

Dr de Leiris

Yes we observed some hearts with some degree of arrhythmias but it was not in all hearts But in these perfusions we used 5 9 mM KCl In another series of experiments I observed that decreasing the concentration of potassium to about 4 or 4 5 mM increased the incidence of arrhythmias in FFA-perfused hearts

FACTORS OF IMPORTANCE FOR THE DEGREE OF ISCHEMIC INJURY IN THE ISOLATED RAT HEART

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SUMMARY

Isolated working rat hearts were made ischemic by introducing a one-way aortic ball valve After the ischemic period the hearts were perfused in a retrograde non-working way for 30 min. Flow rates glycogen, ATP and creatine phosphate went down during the time of ischemia, whereas tissue lactate accumulated. For shorter periods of ischemia these values were normalized but after 30 min of ischemia the hearts seemed to be irreversibly damaged. There was a leakage of GOT GPT LDH and CPK from all hearts when ischemic from 5 to 30 min Different factors that might be of importance for the degree of ischemic injury were tested The injury tended to be more severe at higher heart rates. Addition of adrenaline 10^{-6}M resulted in excessive myocardial damage A variation of pH from 7 1 to 7 7 did not alter the effects of the ischemic injury One group of rats were injected with adrenaline for 8 weeks to simulate chronic stress When hearts from these rats were made ischemic they were more prone to fail compared to controls The failing hearts on the other hand, had a lower leakage of enzymes possibly due to a less severe myocardial damage A high mechanical performance and a normal noradrenaline content of the hearts are key factors for the development of myocardial infarction as indicated by this study

INTRODUCTION

Several techniques have been used to induce experimental myocardial infarction. One of the most used methods involves occlusion of a coronary ar

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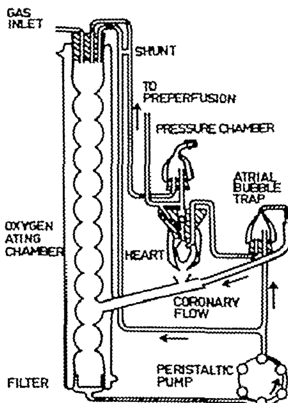


Fig. 1 Perfusion apparatus for isolated working rat heart under ischemic and nonischemic conditions.

Aorta and left atrium are cannulated The one-way ball valve is placed just distal to the coronaries. This ball valve can be bypassed. A Windkessel effect is provided by a partly air-filled chamber connected to the aortic tube The heart is pumping perfusate to the top of the oxygenating chamber A peristaltic pump provides the atrial bubble trap with perfusate The height of this bubble trap is adjustable and the chosen height decides the atrial filling pressure. By clamping the bypass of the one-way valve diastolic perfusion of the coronaries will be inhibited, thus inducing ischemia. (Reproduced with permission from Recent Advances in Studies on Cardiac Structure and Metabolism, University Park Press, Baltimore 1975 Vol 10 (Eds. P E. Roy & G Rona), pp 307 316).

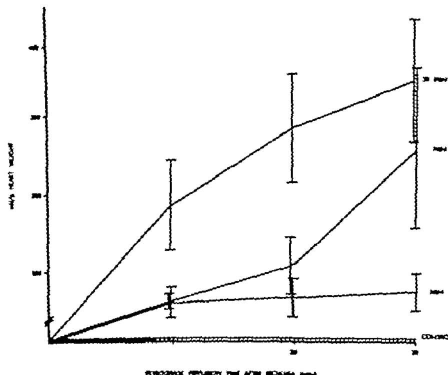


Fig. 5 Release of GOT after different times of ischemia. Hearts were made ischemic for 5 10 or 30 min respectively and thereafter perfused for 30 ml In a retrograde non-working unpaced way Controls were nonischemic throughout the whole perfusion. Each value represents mean ± S.E.M for 6-8 hearts.

is shown in Fig. 3 After 30 min of reperfusion coronary and aortic flows came back to about normal values for hearts previously ischemic for 5 or 10 min When ischemic for 20 min there was some restoration of the coronary flow but the hearts were not producing any aortic flow fter 30 min of ischemia the hearts were so maged that neither coronary nor aortic flow was stored

Another way of illustrating the effects of ischemia is to measure myocardial metabolites (Fig. 4) At the end of ischemia there was a decrease of myocardial glycogen, which was restored again after 30 min of reperfusion for all hearts except those ischemic for 30 min. Myocardial lactate was increased during ischemia but was normalized again for all hearts during the reperfusion period. ATP and creatine-phosphate were decreased during the ischemic period but were built up again during the reperfusion period to approximately normal levels for hearts ischemic for 5 min

A third way of measuring the effects of ischemic

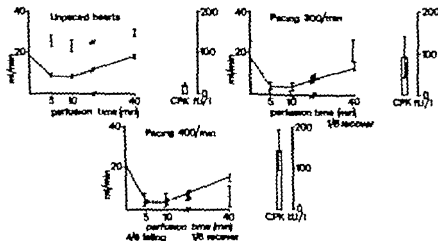


Fig. 6. Effect of heart rate on coronary and aortic flows, and enzyme release All hearts were ischemic for 10 min The heart rate was adjusted by pacing 300/min or 400/min. CPK leakage to the perfusate was taken a parameter for cell damage All values epresent the mean ± S E.M for 6 hearts
—— coronary flow
aortic flow

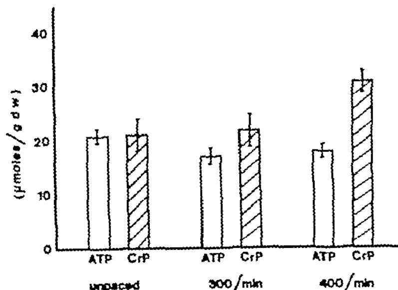


Fig. 7 Effect of heart rate on ATP and creatine phosphate after ischemia (μmoles/g d w) All hearts wer ischemic for 10 min followed by 30 min of retrograde reperfusion (all hearts were unpaced). Each bar represents the mean ± S E.M for 6 hearts.

damage is to analyze the leakage of enzymes from the hearts to the perfusate Fig. 5 shows the leakage of GOT after 10 20 and 30 min of reperfusion. There was a negligible leakage of enzymes from working controls but the longer the ischemic period the more enzymes leaked. Perfusate concentrations of GPT LDH and CPK were also measured at times shown above There was a comparable pattern of leakage for all these enzymes

When the hearts were forced to work during ischemia by pacing to 300 or 400/min coronary flow decreased to about 30 % of the original value and the aortic flow decreased to about 10 % of the original value afte 10 min of ischemia. The eduction of coronary flow for unpaced hearts was only about 50 % while the reduction of aortic flow was as low as 10 % The flow rates for these hearts were restored after 30 min of etrograde perfusion. Most of the hearts in the two paced groups went into failure during ischemia, but there was a good restoration of coronary and aortic flows for hearts unpaced at 300/min. Coronary flow was also fairly well restored for hearts paced at 400/min but there wa poor recovery measured as aortic flow The leakage of CPK from the myocardium to the perfusate was measured after 30 min of eperfusion showing a marked leakage f om the paced ones indicating the negative effect of forced work per during ischemia (Fig. 6)

At the end of the reperfusion period for hearts subjected to 10 min of ischemia there was no difference between the group concerning ATP and eatine-phosphate levels except for a rise of creatine-phosphate for hearts paced at 400/min. This might indicate a block of conversion from creatine-phosphate to ATP (Fig 7).

The importance of local pH for the ischemic damage has been debated (6 7). It was of clinical interest to see whether moderate changes in pH had any influence on heart function and the damage during ischemia. When pH was changed from 7 1 to 7 7 no changes could be found in flow rates during or after ischemia (Fig. 8) The myocardial contents of ATP and creatine-phosphate 30 min after ischemia did not differ between the groups either (Fig. 9)

During the acute phase of myocardial infarction there was an increased adrenergic drive which was believed to be deleterious to the ischemic myocardium (8 9 10 11). When adrenaline 10^{-6}M was added to the perfusate at the start of a 5 min long period of ischemia, there was a marked rise of GOT leakage compared to hearts made ischemic for 5 min without the additional adrenaline (Fig. 10) In fact the effect of adrenaline on these hearts was comparable to hearts made ischemic for 30 min alone The chosen concentration of adrenaline in the perfusate corresponds to a concentration that is believed to occur *in vivo* during extreme conditions. During the early phases of myocardial infarction the local pH is known to be low (7). It has been shown under experimental conditions that endogenous noradrenaline is released during ischemia (8). This situation was mimicked by perfusing hearts that were made ischemic for 15 min at pH 7 1 and at the concentration of adrenaline of $5 \times 10^{-}$ M These hearts were unpaced Fig. 11 shows that coronary and aortic

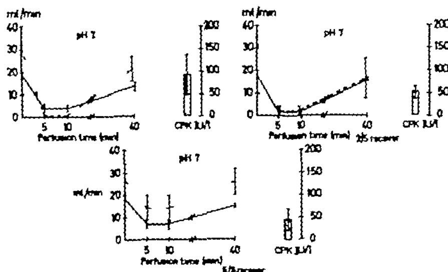


Fig. 8. Effects of pH on flow rates and enzyme release (CPK, IU/l). All hearts wer paced 300/min during ischemic conditions. Each value represents mean ± S.E.M for 6 hearts
——— coronary flow
aortic flow

flows of adrenaline hearts were lower than those of controls. The CPK leakage from adrenaline hearts was significantly higher. The cardioselective beta-blocker metoprolol was added to the buffer of one group of hearts. If the endogenous catecholamine supply is of any importance this effect should be blocked by a beta-blocker. Another way of attacking this problem is to deplete the noradrenaline stores of the hearts by treating the rats *in vivo* by intraperitoneal injections of 6-OH dopamine (12). As can be seen in the lower part of Fig. 11 there were no beneficial effects of the above described treatment as judged by flow rates and CPK leakage when compared to controls. This ay be due to a quick loss of noradrenaline in the control hearts during the preischemic retrograde perfusion resulting in all hearts being noradrenaline depleted at the start of ischemia.

Stress is believed to be a risk factor for developing ischemic heart disease (13, 14). This unwanted effect of stress might mainly either be due to accelerated coronary atherosclerosis or due to the fact that longstanding high blood concentration of catecholamines could metabolically alter the myocardium so that it is more easily damaged by ischemia. One group of rats were injected with adrenaline three times a week for 8 weeks which resulted in a fall in myocardial noradrenaline content to about 50 %. No changes were seen in the contents of noradrenaline when rats were treated with a beta-blocker (metoprolol) for 8 weeks.

When hearts were perfused and made ischemic in presence of adrenaline all hearts failed after 5 min of ischemia and they were still failing after 30 min of reperfusion despite a good coronary flow which is shown in Fig. 12. Some control hearts failed during the ischemic period and others did not. All the failing control hearts got some of the aortic flow back after 30 min of reperfusion. Still the failing controls had a higher enzyme leakage

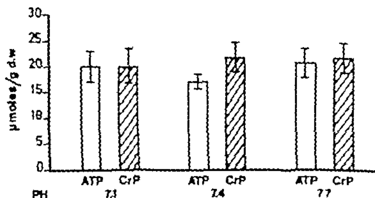


Fig. 9. Effects of pH on ATP and creatine-phosphate (μmoles/g d.w.). All hearts wer ischemic for 10 min and reperfused for 30 min. The hearts were paced 300/min during the time of ischemia. Each bar represent the mean ± S.E.M for 6 hearts.

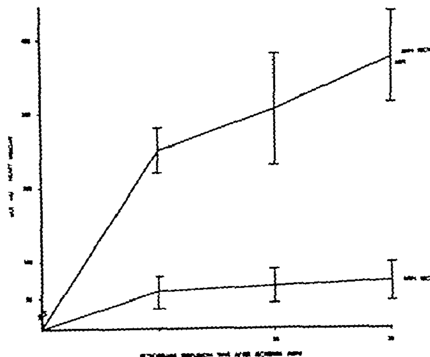


Fig. 10. Release of GOT in presence of adrenaline 10^{-4} M. All hearts in both groups were paced 300/min during the 5 min long time of ischemia. Thereafter they were reperfused in the retrograde non-working way for 30 min (unpaced). Each value represents the mean ± S.E.M. for 6 hearts.

to the perfusate compared to the adrenaline group. In fact the non-failing controls had about the same release of GOT as the adrenaline group. It seems that the noradrenaline-depleted hearts of the long-term adrenaline-treated rats were more prone to go into pump failure when made ischemic compared to ischemic control hearts. The lower leakage of enzymes in these failing hearts could be an indication of a less severe myocardial damage. The myocardial content of GOT from rats pretreated with adrenaline *in vivo* was the same as untreated control which indicates that differences in myocardial content of GOT at the beginning of an experiment cannot be the explanation.

High heart rate induced by pacing and the presence of catecholamines seem to be important factors that induce more severe ischemia and damage

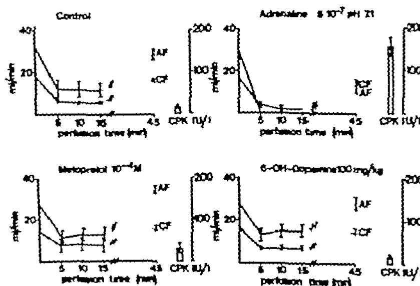


Fig. 11. Effect on coronary and aortic flows and release of CPK in presence of adrenaline at a low pH or metoprolol (selective beta-blocker) on hearts from rats pretreated with 6-OH dopamine. Adrenaline 5×10^{-7} pH 7.1 was chosen to mimic local myocardial conditions at ischemia. Metoprolol was used trying to block the effect of the release of endogenous myocardial noradrenaline. By pretreating animals with 6-OH-dopamine the myocardial stores of noradrenaline are depleted.
CF = coronary flow
AF = aortic flow
——— ischemic perfusion
- - - - nonischemic perfusion
Each value represents the mean ± S.E.M. for 6 hearts.

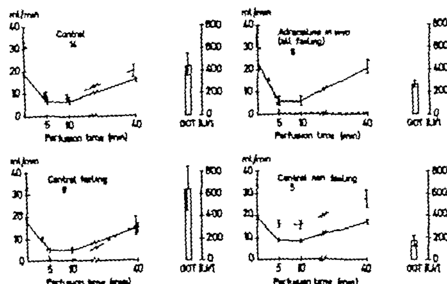


Fig. 12. Enzyme release (GOT mU/mg d.w. × min) and flow rates prior to during, and after ischemia. One group of rats were treated with adrenaline (0.5 mg/kg b.w.) daily for 35 days and paced 300/min during the ischemic period. The controls consisting of 14 hearts were subdivided into one failing and one non-failing group. Each value represents the mean ± S.E.M. for 6-8 hearts. ——— coronary flow ---- aortic flow

to the myocardium in the model system as described above. Moderate changes in pH are not found that important. Reduction of noradrenaline content of hearts by chronic adrenaline overstimulation might protect the heart from ischemic damage and result more likely in heart failure. Unpaced hearts will spontaneously reduce their heart rate and cardiac output during ischemia and these hearts will be protected from marked ischemic damage. It is postulated from this study that a high mechanical performance and a normal noradrenaline content of the hearts are key factors for the development of myocardial infarction.

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DISCUSSION

Dr Morgan

I think that this method of allowing the heart to beat at its spontaneous rate is perhaps the best procedure to get moderate degrees of ischemia in the perfused heart. This is the way in which we intend to pursue studies of more moderate degrees of damage

Dr Waldenström, do you know what cellular changes accompany release of enzymes? Do you think that all the cells that release enzyme are irreversibly damaged? Have you done any histological or electron-microscopic studies on this?

Dr Waldenström

No unfortunately we have not done that We would like to of course. It is difficult to say for sure whether the enzymes can leak out from the cells without the cell being irreversibly damaged, but as glycogen ATP and creatine-phosphate levels are restored after ischemia, it suggests that the cells are still living. But of course it is very difficult to say because there may be an even distribution of dying cells with a compensatory build up in the still living ones. But this seems very unlikely to me

Dr Neely

Most of the enzymes you measured appeared in the perfusat during reperfusion Do you know if release occurs during the ischemic period or during the reperfusion period

Dr Wald nstrom

We have not measured the enzyme release during the ischemic period but only when the hearts are reperfused When we started these experiments we tried to get enough perfusate during the ischemia but this was quite difficult because of the coronary flow

Dr Sobel

Some comments regarding the enzyme release phenomenon in this kind of a model may be appropriate You can certainly produce enzyme loss from skeletal muscle or heart muscle under conditions in which it is not tantamount to cell death That has been done by many investigators including a study by Zierler I believe many years ago with rat diaphragm incubated in calcium-free media. Release of enzyme under such selected conditions appears to be a reversible phenomenon in terms of the function of the tissue. Thus the point that was aluded to is a very important one In a gi en model one cannot interpret what enzyme leakage means until it is related to some other standard As far as reperfusion and the timing of enzyme-release we have performed analogous experiments in which we produced graded intervals of interruption of flow that is reduction of flow followed by restored flow Under these conditions wash-out occurs at the time that the flow increases. Quantitatively more enzyme comes out at that time but when the total amount is integrated it does not necessarily follow that the burst of additional release is correlated with any change in the total amount released It is interesting that there is a morphological correlate to this phenomenon. Damage after reinitiation of flow at least in some models is accentuated as reflected by morphological criteria.

APPLICATIONS AND LIMITATIONS OF ESTIMATION OF INFARCT SIZE FROM SERIAL CHANGES IN PLASMA CREATINE PHOSPHOKINASE ACTIVITY

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INTRODUCTION

Biochemical markers of myocardial infarction have been utilized extensively in detection and in estimation of infarct size. In this presentation our experience with analysis of serial changes in creatine phosphokinase (CPK) in plasma will be reviewed as one particular example of the value and limitations of such biochemical indices (1). Although infarction is traditionally defined by morphological criteria, often they cannot be employed in intact animals and patients. Furthermore because morphological criteria of necrosis evolve slowly they may not provide an accurate index of the extent of injury when survival after infarction is limited. Electrophysiological criteria such as ST-segment elevation in epicardial or precordial recordings are particularly useful in evaluating directional changes in ischemia in individual experimental animals or patients, but absolute estimation of infarct size is difficult (2). Radionuclide imaging techniques have proven most useful in detecting injured myocardium but quantitation of the extent of injury is limited because of superimposition of overlapping regions of myocardium on a two dimensional display poor resolution and limited contrast (3). Positron emission transaxial computer reconstruction tomography offers promise for the future but this technique is not yet available for routine laboratory investigation or clinical use (4).

In part because of the extensive information available regarding changes in plasma enzyme activity accompanying myocardial infarction (5) we felt that analysis of serial changes in plasma enzyme activity would be particularly useful in assessing the extent of ischemic injury. Markers such as CPK seemed particularly advantageous since contrary to the case with lower molecular weight moieties such as myoglobin CPK in plasma is not cleared via the kidney (6). Thus, levels of plasma CPK activity are not dependent on renal blood flow. CPK exhibits additional advantages in comparison with many other enzymes in that its distribution in the heart is confined virtually essentially to myocardial components rather than to connective tissue marginating white cells or other blood elements in contrast to enzymes such as lactate dehydrogenase (LDH) and transaminase (7). The importance of this consideration is underscored by the observation that the total amount of LDH appearing in the blood after myocardial infarction frequently exceeds the total amount of LDH present in the normal heart. Presumably substantial contributions to plasma LDH activity come from blood elements participating in the inflammatory response exhibited by myocardium undergoing necrosis.

THE RELATIONSHIP BETWEEN MYOCARDIAL CPK DEPLETION AND CELL DEATH

Under carefully defined conditions depletion of myocardial CPK activity after experimental myocardial infarction correlates closely with infarct size estimated by independent criteria including the distribution of radioactively labeled microspheres and morphological indices of necrosis (7). Furthermore regional myocardial CPK depletion 4 hours after the coronary occlusion in open chest dogs correlates with the intensity of ischemic injury initially sustained, reflected by ST segment elevation from the same site 15 minutes after coronary occlusion (Fig. 1) (8). Thus it appears that myocardial CPK depletion accompanies and is proportional to ischemic necrosis.

In order to determine whether CPK release is tantamount to cell death in the face of an ischemic insult, we performed a recent series of experiments in conscious and open chest dogs (9). Animals were subjected to coronary occlusion of graded duration from 10 to 60 minutes. Subsequently reperfusion

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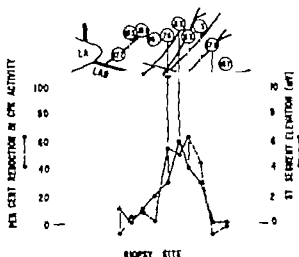


Fig. 1 The relationship between ST-segment elevation 15 minutes after coronary occlusion in an open chest dog and CPK depletion from corresponding regions of myocardium 4 hours later In this typical experiment, branches of the left anterior descending coronary artery were occluded as indicated in the diagram. Epicardial ST-segment elevation is plotted on the right ordinate with open circles and represents changes seen [ ] specific sites 15 minutes after coronary occlusion Twenty-four hours later CPK activity in biopsies from corresponding sites was measured Values obtained are shown in the circles and represent IU/mg protein. The percent reduction in myocardial CPK activity based on activity in non-ischemic myocardium from the same dog is plotted on the left ordinate with the filled circles.

was permitted by release of an exteriorized inflatable coronary artery occlusive cuff Plasma samples were obtained at 30 minute intervals for 12 [ho]urs and hourly intervals for an additional 36 [hour]s at which time the animals were sacrificed and [the] hearts excised The sampling interval was se[lec]ted so that sufficient time would elapse to permit appearance of enzyme in blood after release from ischemic myocardium The 48 hour interval prior to sacrifice was selected to provide sufficient time for evolution of morphological criteria for necrosis At the end of each experiment hearts were sectioned serially and analyzed by conventional histological and histochemical techniques for evidence of necrosis Results indicated that when the duration of occlusion was long (>30 minutes) MB CPK (an isoenzyme found primarily in myocardium) consistently increased in blood exceeding baseline by several fold in each case Similarly all but one of 1 animals with occlusions of this duration exhibited necrosis 48 hours after occlusion On the other hand brief occlusions (≤20 minutes) failed to produce elevated plasma MB CPK activ ty and were not associated with necrosis demonstrable 48 hours later Under these experimental conditions then release of MB CPK into blood and the presence of cardiac necrosis were concordant ($p < .001$). In concert with the substantial clinical and experimental evidence acquired during the past two decades (10-1?) these findings indicate that release of enzyme into blood from myocardium subjected to ischemia reflects cell death

RELATIONSHIPS BETWEEN CHANGES IN PLASMA CPK ACTIVITY AND MYOCARDIAL CPK DEPLETION

Acute myocardial infarction leads to characteristic serial changes in plasma CPK activity both in experimental animals and patients Accordingly we attempted to estimate myocardial CPK depletion from the serial changes in plasma CPK Since myocardial CPK depletion appeared to be a quantitative index of the extent of infarction it appeared likely that estimates of myocardial CPK depletion obtained in this fashion could provide an index of infarct size applicable to intact experimental animals or patients The initial formulation of the approach assumed a simplistic model in which the rate of change of CPK enzyme activity/unit time (dE/dt) was assumed to be determined by two competing phenomena. 1) release of CPK from the heart, f(t) and) disappearance of CPK from blood assumed to conform to first order kinetics with a constant fractional disappearance rate k_d (13) In experimental studies k_d was estimated from serial changes in plasma CPK after injection of purified canine myocardial CPK intravenously in conscious dogs In patients k_d has recently been estimated from serial changes in plasma after CPK release from the heart has presumably ceased after acute myocardial infarction (14). In the initial formulation cumulative CPK release ($\int f(t)dt$) was calculated from measured serial changes in plasma CPK activity simply by rearrangement of terms and integration (13). Calculations and constants currently used in our laboratory are summarized in the Appendix (6) In order to estimate infarct size from cumulative CPK release several parameters are utilized including the distribution volume of the enzyme the amount of CPK in normal myocardium and the amount remaining in myocardium undergoing homogenous infarction These parameters have been evaluated in experimental animals in which myocardial CPK depletion was measured directly (6 13 15)

In our initial experiments, we found that the correlation was close between cumulated CPK released into plasma and the amount of CPK lost from myocardium in conscious dogs subjected to experimental coronary occlusion (Fig. 2) (13) It should

Table 1 The Disappearance Rate (k_d) of MB CPK and Total CPK from the Circulation in Twelve Consecutively Studied Patients with Acute Myocardial Infarction.

	Total CPK	MB CPK
Average k_d (min^{-1})	1 1 (0 6 to –0) × 10	1 7 (1.– to 2.3) × 10
Average Standard Deviation of k_d (expressed as % of k_d)	9 (4 to 15) %	8 (2 to 12) %
Correlation coefficient (r)	0.97 (0 95 to 0.99)	0.97 (0.95 to 0 99)

k_d was calculated as described by Norris (14) from the descending portions of MB and total serum CPK curves Ranges are indicated in parentheses. r = the correlation coefficient of the last regression line (least squares method) used to calculate k_d from the descending portion of the curve relating the log of CPK activity to time

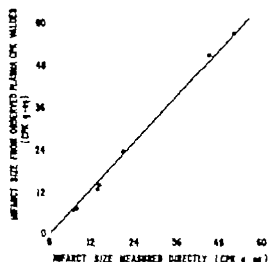


Fig. 2 The relationship between infarct size estimated from serial changes in plasma CPK activity in conscious dogs subjected to coronary occlusion and infarct size estimated by direct measurement of myocardial CPK depletion in the same animals. As can be seen, the estimates of cumulative CPK released calculated from serial changes in plasma CPK activity corresponded closely to infarct size estimated from myocardial CPK depletion measured directly

be emphasized that the experimental model employed was selected specifically to avoid interference from noncardiac CPK released into blood as the result of a surgical procedure Thus coronary occlusion was performed in conscious ambulatory animals by constriction of a previously placed exteriorized occlusive cuff around the left anterior descending coronary artery The particular vessel occluded was selected to avoid the marked hemodynamic impairment associated with occlusion of the circumflex coronary artery in dogs likely to lead to release of noncardiac enzyme from hypoperfused tissues in the periphery

We have recently evaluated the extent to which CPK disappearance conforms to first order kinetics both in experimental animals and patients (6) As can be seen in Table 1 CPK disappearance is first order with a high correlation coefficient. Furthermore as shown in Table hemodynamic perturbations simulating changes seen in association with acute myocardial infarction do not alter CPK disappearance rates appreciably (6)

In order to determine whether k_d remained constant from day to day CPK disappearance was evaluated in conscious dogs after sequential daily intravenous injections of purified canine myocardial

Table 2 The Effects of Hemodynamic Alterations on the Disappearance Rate of CPK from the Circulation.

Hemodynamic Intervention		(k_d) Before Intervention/After Intervention	
Diminution of cardiac output by > 90 % by constriction of the inferior vena cava	4	0 99	(0.9? to 1 04)
Heart rate accelerated from < 100 to 180 beats/min	8	1.08	(1 02 to 1 12)
Unilateral (n=2) or bilateral (=) artery occlusion	4	1 01	(0.94 to 1 06)
Hepatic artery occlusion	5	0.9?	(0.87 to 1 0)
Inhibition of the reticuloendothelial system by administration of Zymosan, 10 mg/kg	3	5.3	(4 4 to 6 6)

Results expressed are means with ranges indicated in parentheses.

*k_d = The fractional disappearance rate of CPK from the circulation, calculated from the slope of the best fit regression line (least squares method) of the log of CPK activity vs. time A theoretical ratio of 1 would indicate no change in k_d after the intervention As can be seen, despite profound hemodynamic perturbations simulating those seen with hemodynamically complicated acute myocardial infarction, k_d varied by less than 10 %

Table 3 CPK Disappearance Rates (k_d) Determined Repetitively in Conscious Dogs.

	Fractional CPK Disappearance Rate (k_d) (min^{-1})			
Dog Number	Day 1	Day 2	Day 3	Mean
1	4.3×10^{-3}	4.1×10^{-3}	4.4×10^{-3}	4.1×10^{-3}
2	5.6×10^{-3}	5.2×10^{-3}	5.0×10^{-3}	5.3×10^{-3}
3	6.2×10^{-3}	5.7×10^{-3}	6.3×10^{-3}	6.3×10^{-3}
4	4.9×10^{-3}	5.4×10^{-3}	5.6×10^{-3}	5.3×10^{-3}
5	3.8×10^{-3}	4.2×10^{-3}	3.9×10^{-3}	4.0×10^{-3}

Purified canine CPK was injected intravenously in the same conscious dog on three successive days in each case and k_d was calculated from hourly changes in serum CPK activity

CPK In experiments performed with radioactively labeled material or with cold enzyme the variation of k_d in the same animal from day to day was consistently less than 10 % (Table 3) (16) Comparable results have been obtained in patients with spontaneous extension of myocardial infarction in whom repetitive determinations of CPK disappearance were possible (14)

In order to determine whether the presence of myocardial infarction *per se* alters CPK disappearance studies were performed in conscious dogs with exteriorized coronary artery occlusive cuffs (16) CPK disappearance was measured directly by intravenous injection of cold enzyme several days prior to the experiment Subsequently experimental myocardial infarction was induced and while enzyme was still being released from the heart, ^{14}C-labeled CPK (prepared with ^{14}C formaldehyde and sodium borohydride (17)) was injected intravenously Under these conditions the decline in counts/min/ml blood with time provides an index of the disappearance of exogenous enzyme even though total plasma enzyme activity may still be increasing because of release of endogenous enzyme from the heart The decrease of specific activity of the tracer is inversely proportional to augmentation of pool size and hence the rate of decline of tracer radioactivity in blood would not change as long as k_d remained constant. As can be seen in Fig. 3 CPK disappearance remains constant in the conscious dog despite interceding myocardial infarction

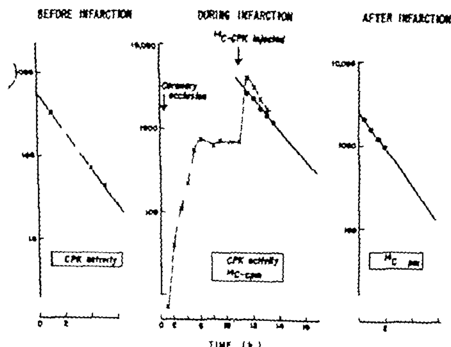


Fig. 3 Calculations of CPK disappearance rate under three conditions in the same dog. In the left panel CPK disappearance rate was obtained from the measured decline in plasma CPK activity after intravenous injection of purified canine myocardial CPK. In the middle panel, disappearance rat was estimated from the serial changes in radioactive counts associated with ^{14}C-CPK, purified from canine myocardium and radioactively labeled with ^{14}C-formaldehyde after intravenous injection of the material in a consciou dog undergoing experimentally induced coronary occlusion. I the panel on the right, the disappearance rate of the adioactively labeled material was measured again several days later in the same animal after baseline CPK acti ity had eturned to normal A can be seen by the slopes of the three lines CPK disappearance emained quite constant despi inter edent myocardial infarction

Table 4 The Effects of Pharmacological Interventions on CPK Disappearance Rate

Intervention	Number of Animals Studied	Dose	Fractional Disappearance Rate ($\times 10^{-3} min^{-1}$) Control	Intervention	Percentage change in CPK Disappearance Rate
Nembutal	3	30 mg/kg	4 0	[illegible]	−50.0
			5.3	2.2	−58.4
			6.2	3 4	−51.6
					mean −52.0 %
Lidocaine	3	4 mg/kg bolus injection + 2 mg/kg q 30 min	4 7	4 7	0
			5.2	5 4	+ 3.8
			6 3	6.3	0
					mean + 1.[illegible] %
Methylprednisolone	3	30 mg/kg	6.3	6.0	− 4 7
			4 9	5.0	+ 2.0
			4 4	4 4	0
					mean + 0 9 %
Morphine	3	2 mg/kg q 30 min	4.6	2.6	−43.4
			5 7	3 1	−45.6
			4 9	2.2	−55 1
					mean −48 0 %
Morphine	3	0.2 mg/kg q 60 min	4 1	3 7	− 9 7
			3 9	3 4	−12.8
			6 7	6 2	− 7 4
					mean − 9 9 %
Valium	3	1 mg/kg q 60 min	6.7	3.6	−46.2
			7 2	4 1	−43 0
			5 7	2 8	−50 8
					mean −46.3 %
Valium	3	0.1 mg/kg	5.8	5.2	−10.3
			6.4	6.1	− 4 6
			5 2	4 7	− 9 6
					mean − 8.3 %

These results indicate 1) disappearance of CPK conforms remarkably closely to first order kinetics 2) CPK disappearance is not influenced significantly by profound hemodynamic perturbat ons 3) CPK disappearance does not vary appreciably from day to day in the same conscious animal or patient and 4) intercedent myocardial infarction does not change CPK disappearance substantially

As shown in Table 4 pharmacological and meta bolic interventions may alter CPK disappearance dramatically We have found that anesthesia or ad ministration of large doses of Valium, sodium pentobarbital or morphine inhibit the clearance of CPK from the circulation and cause disappearance to virtually cease (16) Accordingly estimation of infarct size from analysis of serial changes in plasma CPK activity requires consideration of the pharmacological and metabolic environ ment during the study Since Zymosan, a known inhibitor of activity of the reticuloendothelial system also inhibits disappearance of CPK from the circulation (6) one of the factors responsible for removal of enzyme may be activity of this system.

One other parameter involved in the model initially formulated is the CPK released/depleted ratio – i.e the ratio of CPK appearing in blood to that lost from myocardium undergoing infarction. As noted in the Appendix the value used for this ratio is 15 % based on the empirical observations relating serial plasma CPK changes to myocardial CPK depletion measured directly in conscious dogs In order to explore possible reasons for the low value of this ratio one must consider the mechanisms by which CPK is transported from the heart into the circulation.

Since coronary flow is limited within an infarct and since Malmberg and others have shown that enzyme may be transported via the lymph (18) we examined the effects of lymph on CPK activity *in*

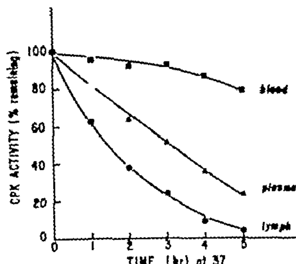


Fig. 4 The effects of exposure to dog lymph, plasma, and blood on CPK incubated *in vitr* Under conditions in which pH in the three media wa maintained constant at 7.8 and an equivalent amount of heparin was added to plasma and lymph so that no differences in anticoagulant concentration were present the rate of decline of CPK activity was substantially greater when samples were incubated in lymph compared to plasma or whole blood. Inactivation in lymph could be precluded by adequate fortification with thiol groups. Inactivation was accentuated by deproteinization of lymph prior to incubation and wa diminished by dialysis consistent with removal of an oxidant by this process.

vitro and *in vivo* Under conditions in which temper ature was maintained at 37° and pH held constant activity of CPK (but not LDH) decl ned substantial ly more rapidly in lymph than plasma or blood (Fig 4) For example 50 % of act vity was lost in 76 minutes when enzyme was incubated in lymph compared to 660 minutes when it was incubated in whole blood Similar results were obtained when enzyme was incubated in lymph *in situ* The loss of activity was not due to proteolysis detectable by polyacrylamide gel electrophoresis with gels stained for enzyme and protein independently (19) Loss of CPK activity was associated with diminu tion of titratable f ee sulfhydryl groups and protec tion wa conferred by addition of dithioth eitol Loss of CPK acti ty in lymph w precluded by dialysi but not by deproteinization These results indicate that CPK activity is lost rapidly in lymph *in vitro* and *in situ* because of thiol oxidation Accor dingly if CPK transported in lymph f om ische mic myocardium to the systemic blood ci culation one would anticipate substantial los of ct ity particularly because lymph flow so low en afte myocardial infa ction and hence the duration of ex posure of the enzyme to the deleteriou en on ment would be prolonged Thu the k w leased/depleted ratio nd it lack of ariati n over a wide range of infarct size may be due to a predominant effect of substantial inactivation of CPK in lymph These observations under score the importance of consideration of phy siological processes governing release of bio chemical markers from ischemic myocardium and their disappearance from the circulation (20 21)

In order to improve estimates of infarct size based on analysis of enzyme changes we sought to utilize the MB CPK isoenzyme (6) Conventional assays employing electrophoresis and fluorometric scanning are not adequate for quantitative estima tion of activity in blood samples (22). We recently developed a batch adsorption procedure to over come these difficulties (23) and used it to survey a large number of human tissues obtained freshly at surgery (24) *Fortuitously we found that in man,* contrary to the dog MB CPK is confined virtually exclusively to the heart (Fig. 5) None was detected in skeletal muscle brain gastrointestinal tract, kid ney liver spleen thyroid prostate or washed red cells under conditions in which less than 1 % would have been readily detectable by the assay em ployed The proportion of MB CPK found in myo cardial extracts was 15 % of total CPK a value comparable to that obtained with chromatographic methods by others but substantially less than esti mates based on qualitative electrophoretic scan ning procedures

Use of serial changes in MB CPK activity in plasma samples after myocardial infarction is likely

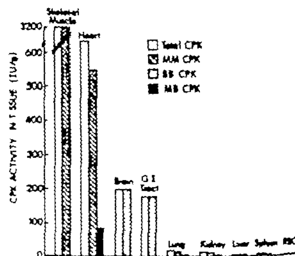
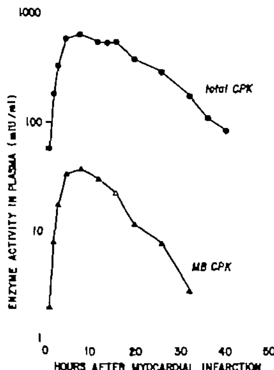


Fig 5 The distribution of CPK isoenzymes in tissue act obtained f sh at surgery from a minimum of three patient in ach case A can be seen among the tissue (xamined MB CPK was detected only in xtract of my ocardium under onditions in which less than 1 % would ha been det d by the ssay utilized



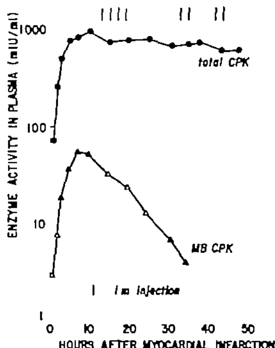


Fig. 6. Serial changes in total and MB CPK activity in plasma in patient with hemodynamically uncomplicated acute myocardial infarction. As can be seen, MB CPK activity declined somewhat more rapidly than total CPK but the general shape of the two curves was similar.

Fig. 7. Changes in MB and total CPK in serial plasma samples from patient with acute myocardial infarction given intramuscular injections at the times indicated by vertical lines at the top of the figure. The distortion in the total plasma CPK curve appears to reflect release of enzyme from skeletal muscle. In contrast, the MB CPK curve remains typical of that associated with acute myocardial infarction.

to improve estimates of infarct size (6). After infarction total CPK activity increases and MB CPK activity in blood increases reaching approximately 15% of peak activity as shown in Fig. 6. When endogenous noncardiac enzyme is released into blood after intramuscular injections the serial changes in total CPK are distorted but the MB CPK curve continues to reflect the characteristic changes indicative of uncomplicated acute myocardial infarction (Fig. 7). A similar lack of distortion of the MB CPK curve is evident in patients with noncardiogenic shock in which persistent plasma CPK elevations do not reflect persistent myocardial necrosis.

The specificity of elevated MB CPK activity in plasma as an index of myocardial infarction was examined in a recent study of patients undergoing noncardiac surgery. Among 100 patients undergoing thoracic, abdominal, orthopedic, genitourinary tract and gastrointestinal tract surgery none exhibited plasma MB CPK elevations in any of serial samples obtained prior to surgery and for 4 hours subsequently (Fig. 8). Similarly among 53 patients undergoing cardiac catheterization none exhibited elevated MB CPK in serial samples obtained for a

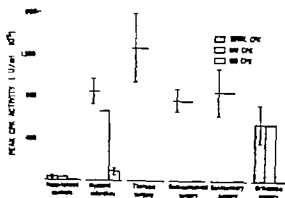


Fig. 8. Peak total MM and MB CPK activity in plasma from 90 hospitalized controls, 100 patients with acute myocardial infarction and 100 patients undergoing thoracic, gastrointestinal tract, genitourinary tract and orthopedic surgery. As can be seen, the only group of patients with elevated MB CPK activity were those with acute myocardial infarction. In these studies samples were obtained at hourly intervals for six hours and six hourly intervals for an additional 18 hours in each case after the event being studied.

minimum of 24 hours (24). The absence of MB CPK elevations contrasts with the virtually universal elevation of total plasma CPK activity among patients studied after noncardiac surgery and the frequent (>30 %) elevation of total plasma CPK activity in patients undergoing diagnostic cardiac catheterization Thus trauma to tissues other than the heart does not lead to elevated plasma MB CPK activity and hence would not obscure the diagnosis of acute myocardial infarction As we and others have noted patients with acute myocardial infarction virtually invariably exhibit elevated MB CPK activity averaging 10 to 15 % of total plasma CPK (25-27)

Based on these findings we recently estimated infarct size enzymatically from serial changes in plasma MB CPK activity in patients with myocardial infarction (6) Patients selected were those without hemodynamically complicated infarction so that enzymatic estimates based on total plasma CPK would be least likely to be affected by noncardiac CPK Estimated infarct size based on analysis of total CPK ranged from 12 to 187 CPK-g-eq The relation between estimates of infarct size based on MB (IS_{MB}) and total CPK (IS_{total}) conformed to a regression line: IS_{total} = (99) (IS_{MB}) + 1 8 with a standard deviation of the slope of the best fit line =

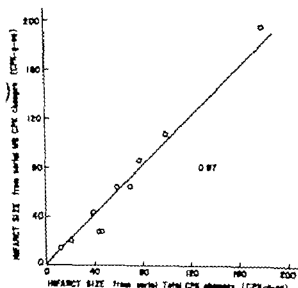


Fig. 9 The relationship between infarct size estimated from serial changes in plasma MB CPK activity compared to that estimated from serial changes in total CPK activity in patients with acute myocardial infarction A can be seen the correlation between the two estimates was close despite the fact that the disappearance ate of MB CPK often differed substantially f om the disappearanc ate of total CPK. For both estimates disappearance ates ere calculated from the terminal portions of nzyme urves after release had presumably ceased (6, 14)

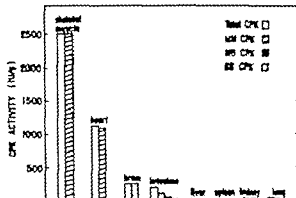


Fig 10 The distribution of CPK isoenzymes in tissue extracts from dogs. Assays were performed under standardized conditions in which <1 % of each isoenzyme could be readily detected in the extract. Results represent averages from at least three animals in each case In contrast to findings with qualitative techniques, the proportion of MB CPK in heart muscle is relatively small. In contrast to man (Fig. 5) the proportion of MB in gastrointestinal tract exceeds that in extracts from the heart. Thus MB CPK is a less specific marker of myocardial injury in the dog than in man Because the proportion of MB is so small in canine myocardium, its contribution to the disappearance rate of CPK after experimentally induced myocardial infarction is probably modest.

07 and r = 97 Thus despite the markedly different disappearance rates of MB and total CPK, estimates of infarct size from serial changes in plasma total CPK and MB CPK correlated closely (Fig. 9)

In our initial attempts to estimate infarct size from total plasma CPK changes we verified results in conscious dogs by measuring myocardial CPK *depletion directly and comparing it to estimated* cumulated CPK release based on analysis of changes in serial plasma samples. Unfortunately the proportion of MB CPK in dog myocardium is so low (less than 1 %) (Fig. 10) that analogous experiments with the MB isoenzyme are prone to a large percentage error because of the high *background total CPK in the canine heart.* Accordingly we elected to compare estimates of infarct size based on MB CPK to estimates in patients with hemodynamically uncomplicated infarction in whom serial changes in plasma CPK activity could be used as a reasonable index of infarct size

CORRELATIONS BETWEEN INFARCT SIZE ESTIMATED ENZYMATICALLY AND OTHER INDEPENDENTLY MEASURED PARAMETERS

Enzymatic estimation of infarct size has proven useful n ev luating patients with acute myocardial

technique for individualization of the parameter k_d used to estimate infarct size in specific patients (14). As noted previously, CPK disappearance from the circulation appears to conform to first order kinetics and to be uninfluenced by intercedent myocardial infarction or profound hemodynamic derangements, but enzymatic estimates must take into account the possible effects of pharmacological agents used to treat patients in the clinical setting. Improved enzymatic estimates appear to be possible with the use of the MB CPK isoenzyme, a more specific marker of myocardium than total plasma CPK activity. With the availability of convenient quantitative techniques for estimation of plasma MB CPK activity, it should be possible to obtain accurate enzymatic estimates of infarct size even in patients with enzyme release from noncardiac sources such as those subjected to intramuscular injections (33) or hypoperfusion of visceral organs and skeletal muscle which can result in release of noncardiac CPK. Improved estimates may also result from models being developed describing physiological processes governing release of CPK from the ischemic heart and its transport into the circulation (20, 21).



Fig. 11. The relation between infarct size estimated from serial changes in plasma CPK activity in 58 patients admitted sufficiently early so that initial activity was <100 mIU/ml. Mortality figures refer to death within one month after onset of infarction. The overall mortality of the group was 21%. Patients with infarct size <50 CPK-g-eq exhibited an early mortality of only 3% compared to 12 fold greater mortality in patients with infarct size of 50 CPK-g-eq or more ($p < .001$). IS refers to infarct size.

infarction. The six month mortality of patients with large infarcts is substantially greater than mortality in those with small infarcts (Fig. 11) estimated by this technique (28). Alterations of ventricular compliance (29), impairment of ventricular function (30), early clinical manifestations of infarction (14), and the frequency and severity of ventricular dysrhythmias during the first 10 hours after hospital admission (Fig. 12) (31) all correlate with infarct size estimated enzymatically. Enzymatically estimated infarct size correlates closely ($r = .98$) with infarct size estimated histochemically in patients dying relatively soon after the onset of myocardial infarction but after an interval sufficiently long for analysis of serial changes in plasma CPK activity (32).

Despite the clinical utility of enzymatic estimates of infarct size, continued refinement of the method should prove useful in reducing the variance of estimates. For example, Norris has proposed a

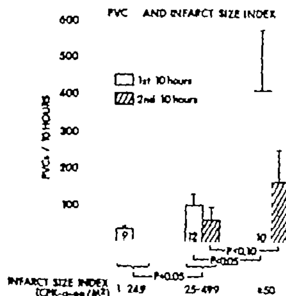


Fig. 12. The relation between ventricular dysrhythmia and infarct size index (CPK-g-eq/m² body surface area). Premature ventricular complexes (PVCs) were quantified with the use of an Argus/H computer system by analysis of continuous electrocardiographic tape recordings obtained during the first and second 10 hour intervals after admission of each patient. As can be seen, the frequency of PVCs during the first 10 hours was directly related to infarct size index. A similar trend was evident when PVC during the second 10 hours after admission were compared in patients with increasing infarct size index.

PREDICTION OF INFARCT SIZE FROM SERIAL PLASMA CPK CHANGES

In order to determine whether the evolution of myocardial infarction is dynamic and whether its ultimate extent could be modified by early interventions designed to reduce myocardial oxygen re quirements we sought to predict infarct size on the basis of projected serial changes in plasma CPK activity *Initially projections were made entirely* empirically In other words observed changes of plasma CPK activity were curve fit by least squares approximation to an arbitrarily selected mathematical algorithm the lognormal function, chosen simply because the observed data from a series of conscious dogs conformed closely to lognormal curves with parameters selected on the basis of least squares approximation (15) Entire data sets from individual animals and patients fit lognormal curves well. In addition, projections of plasma CPK values were obtained based on best fit curve derived from serial changes during the first five *hours (dogs) or first seven hours (patients) after* the initial plasma CPK elevation. In a series of conscious dogs subjected to constriction of the left anterior coronary artery projected CPK values correlated closely with observed values and infarct size estimated from all plasma CPK changes correlated closely (r = 96) to infarct size predicted i e calculated from the projected plasma CPK values (15)

Although we have utilized the empirical algorithm in several experimental and clinical studies (see below) we have recently attempted to improve the accuracy of projected plasma CPK values by developing physiologically based model i e one utilizing parameters representing physiological processes governing elease of CPK from ischemic *myocardium nact ation or degradation locally* transport to the ystemic vascular circulation and disappearance of enzyme activity from blood (20 21) Empirical and physiologically based models differ in the same w y as projections of stock mar ket prices based on technical considerations e the empirical behavior of the market in the past compared to those based on forces in the economy (parameters) influenc ng the behavior of the market as a whole In prelim nary tudies of physiologically based models utilizing serial plasma CPK changes we have found that parameter estimates obtained with the model conform quite closely to ndependently measured values of selected parameters such as thickness of the left entricular wall

Results obtained with the empirical approach indicated that infarct size could be modified dur ng t early evolution in conscious dogs subjected to coronary occlusion. Thus reperfusion five hours after the initial plasma CPK elevation resulted in salvage of myocardium in one group of dogs but unexpected and morphologically verifiable extension of infarc tion associated with myocardial hemorrhage in another (Fig. 13) (34). Acceleration of heart rate within 7? hours after the onset of myocardial infarc tion led to augmentation of myocardial CPK release indicative of extension (Fig. 14) presumably reflect ing the deleterious effects of increased myocardial

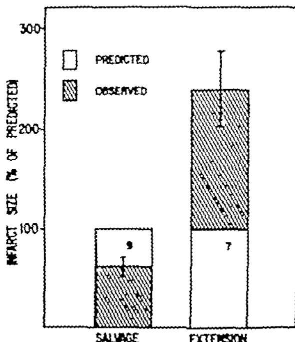


Fig. 13 Effects of reperfusion on infarct size in conscious dog. After xperimental coronary artery occlusion produced by constriction of an exteriorized snare in conscious dogs, projected CPK values were obtained from the best fit curve conforming to elevated values obtained during the first five hours after the initial plasma CPK elevations. These projected values were used to estimate predicted infarct size Observed infarct size was calculated from CPK values prior to reperfusion as well as those observed after eperfusion initiated as soon as the projected curve had been obtained A can be seen, in nine dogs observed values after eperfusion were substantially lower than projected values and hence observed infarct size was substantially less than that predicted indicative of salvage of myocardium resulting from reperfusion On the other hand, in the remaining seven dogs massive elevations of plasma CPK activity followed reperfusion giving rise to observed infarct size values markedly xceeding predicted infarct size estimates The presence of ma siv hemorrhage in the heart of those animals exhibiting enzymatically detectable extension of infarction suggest that the results reflect a disparate effect of eperfusion on survival of myocardium rather than merely an alteration in the plasma CPK curves induced artifactually by reperfusion. Hemorrhage w not present in myocardium of dogs hibiting salvage

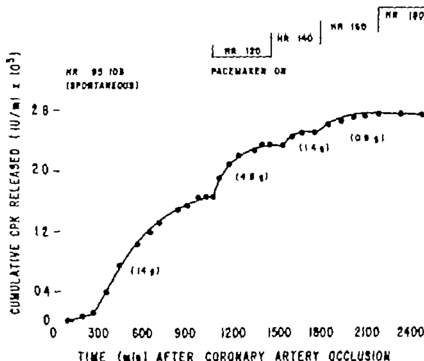


Fig 14. Detection of deleterious effects of acceleration of heart rate on infarct size in conscious dogs. In this representative experiment, experimental myocardial infarction was induced in a conscious dog by constriction of an exteriorized coronary artery snare. After the evolution of infarction appears to be complete judging from flattening out of the initial cumulative CPK release curve, heart rate was accelerated by ventricular pacing in a series of steps. As can be seen, this intervention led to augmentation of CPK release into plasma with calculated extension of infarction in CPK gram-equivalents (g) shown in parentheses.

oxygen demands (35). Reduction of myocardial oxygen requirements by administration of propranolol led to diminished release of CPK into the circulation compared to that anticipated on the basis of projected curves obtained prior to administration of the drug (36). On the basis of these and other findings, it appeared likely that physiological and pharmacological interventions that reduced myocardial oxygen requirements during the early evolution of infarction resulted in salvage of tissue. Accordingly, a series of studies was performed in patients using the same technique.

In 14 patients with acute myocardial infarction and hypertension at the time of admission, trimethaphan was administered intravenously for 24 hours to reduce peripheral arterial resistance, lower ventricular afterload, and reduce myocardial oxygen requirements. In each case, projected plasma CPK values were obtained from the best fit lognormal curve conforming to plasma CPK changes during the first seven hours after the initial elevation. The intervention was implemented immediately thereafter and subsequent observed plasma CPK values were compared to those projected. Observed infarct size was estimated from actual plasma CPK values before and after the intervention and compared to infarct size predicted from the projected values. In the group as a whole, observed infarct size was significantly less than infarct size predicted indicating protection of myocardium and reduction of enzymatically manifested ischemic injury (Fig. 15). On the other hand, in two groups of controls (one matched for blood pressure and the other for predicted infarct size) values of observed and predicted infarct size did not differ significantly (37).

We have recently examined several other interventions including external pressure circulatory assist, administration of Dobutamine, and administration of methylprednisolone in patients with evolving myocardial infarction. Although external pressure circulatory assist reduced ventricular dysrhythmia transiently, it had no net effect on the relation between observed and predicted infarct size (38). In patients with impaired ventricular performance, it is often necessary to increase cardiac output both to maintain coronary perfusion and protect visceral organs against ischemic damage. Unfortunately, agents conventionally used for this purpose extend myocardial infarction in the experimental animal. Cardioselective beta adrenergic agonists such as Dobutamine, relatively devoid of positive chronotropic effect, offer the potential advantages of improving cardiac performance without accelerating heart rate markedly, diminishing ventricular afterload by dilating the peripheral arterial bed, and thereby improving the balance between myocardial oxygen requirements and oxygen supply

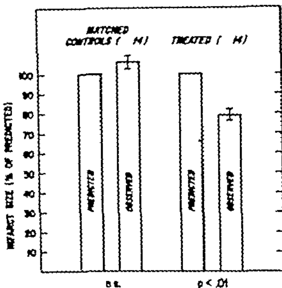


Fig. 15 The effects of reduction of ventricular afterload in patients with hypertension at the time of admission associated with acute myocardial infarction. In controls matched for predicted infarct size observed and predicted infarct size were comparable. However in patients treated with trimethaphan to reduce systemic arterial blood pressure and lower myocardial oxygen requirements observed infarct size calculated from observed plasma CPK changes before and after the intervention was significantly less than predicted infarct size estimated from best fit curves conforming to plasma CPK changes prior to implementation of the intervention.

and at the same time enhancing ventricular performance. In patients studied recently with depressed cardiac output administration of Dobutamine resulted in favorable hemodynamic effects without augmenting the extent of myocardial injury detectable from analysis from serial plasma CPK changes. Accordingly Dobutamine seems to be a relatively safe cardiotonic agent for use in the clinical setting of evolving myocardial infarction (39).

Our studies with methylprednisolone were stimulated by experimental findings suggesting that this agent protects ischemic myocardium presumably by stabilizing lysosomal membranes (40). Under experimental conditions pretreatment with corticosteroids reduces electron microscopically detectable morphological damage in ischemic tissue (41). Administration of corticosteroid to patients appeared to protect ischemic myocardium as well (42). For these reasons we administered 30 mg/kg methylprednisolone intravenously seven hours after the initial plasma CPK elevation as a single dose to one group of patients and in multiple doses (every six hours for 48 hours) to a second group. Patients in both groups were compared to controls matched for predicted infarct size (43). No significant difference was demonstrable between observed and predicted infarct size in controls or patients given a single dose of methylprednisolone. However marked extension of infarction ($p < .001$) was evident in the patients given methylprednisolone in multiple doses (Fig. 16). The deleterious effects of methylprednisolone administered in this fashion were confirmed by persistence of MB CPK elevations in plasma well beyond the time when MB CPK activity had returned to normal in controls or patients given a single dose of methylprednisolone. The apparent extension of infarction was associated with an increase in ventricular dysrhythmia in patients treated with methylprednisolone compared to a decrease during the corresponding time interval in controls. Two of the 12 patients treated with multiple dose methylprednisolone succumbed with ventricular rupture verified at necropsy. The deleterious effects observed may be related to delayed healing and impaired scarred formation (44).

These experimental and clinical findings suggest that physiological and pharmacological interventions are capable of modifying the evolution of acute myocardial infarction. Although enzymatic estimation of infarct size has proven useful in establishing the dynamic nature of acute myocardial infarction, currently available technique suffers from one very important limitation. Thus, implementation of an intervention to be studied must be delayed for seven hours (in man) after the initial plasma CPK elevation in order to provide a sufficient interval for collection of data from which best fit curves used to project subsequent CPK values can be obtained. This delay may mask the efficacy of the intervention because interventions are most likely to be efficacious when implemented as soon as possible after the onset of infarction. Our current efforts in this area are designed to reduce the interval required for adequate projection by utilizing serial changes in plasma MB CPK rather than total CPK activity (6) and developeng a physiologically based model taking into account processes governing release transport and clearance of MB CPK rather than relying on an empirical algorithm for projecting serial changes in plasma enzyme activity (20, 21). Another approach being explored actively utilizes an independent means positron emission transaxial computer reconstruction tomography for assessing the amount of myocardium in jeopardy soon after admission of a patient to the hospital and comparing ultimate infarct size estimated enzymatically to an

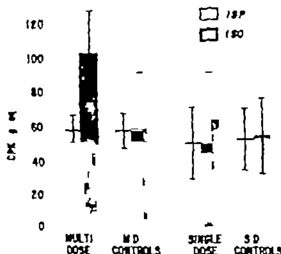


Fig. 16. The effects of single and multiple doses of methylprednisolone on enzymatically estimated infarct size in patients with acute myocardial infarction. Each group comprises 12 patients. Controls were matched for predicted infarct size to both multiple dose methylprednisolone and single dose methylprednisolone treated patients. As can be seen, the only group exhibiting disparity between predicted and observed infarct size was patients treated with multiple doses of methylprednisolone in whom observed infarct size exceeded that predicted prior to the intervention significantly ($p < .01$)

initially jeopardized zone exhibiting diminished uptake of cyclotron produced ^{11}C-palmitic acid (45)

CONCLUSION

Results of enzymatic estimation of infarct size have suggested that the extent of infarction is an important determinant of prognosis. In addition, it appears to underly the severity of impairment of ventricular function the frequency and severity of early ventricular dysrhythmia, and the occurrence of many clinical manifestations. Enzymatic estimates correlate with morphological estimates of infarct size in patients and independent criteria of the extent of damage in experimental animals. Improved estimates should result from exclusion of noncardiac CPK development of physiologically based models describing release transport, inactivation, and clearance of CPK from the circulation and individualization of estimates of CPK disappearance rates. The lack of dependence of the rate of disappearance of CPK from the circulation on hemodynamic perturbations or myocardial infarction *per se* has been documented. Use of projected enzyme values has helped to document the dynamic nature of evolving myocardial infarction and its susceptibility to favorable modifications by appropriate physiological and pharmacological interventions

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APPENDIX

Calculation of Infarct Size from Serial Changes in MB or Total CPK (expressed as CPK-gram-equivalents)

1 E(t) – activity of CPK in blood (IU/ml)

f(t) – rate of change of CPK activity due to enzyme being released by heart (IU/(ml min))

k_d – fractional rate of disappearance of CPK from blood (min^{-1})

$$\frac{dE}{dt} = f(t) - k_d E$$

2 CPK_T – cumulative activity of CPK released by heart up to time T (IU/ml)

$$CPK_T = \int_0^T f(t)\,dt = E(T) + k_d \int_0^T E(t)dt$$

Note that CPK_T is a function of T

3 K – proportionality constant $\left(\frac{(ml)\,(CPK\text{-}g\text{-}eq)}{(kg)\,(IU)}\right)$

DV – distribution volume/unit body weight $\left(\frac{ml}{kg}\right)$

P_{CPK} – proportion of CPK released into blood compared to CPK depleted from the heart (IU/CPK-g-eq)/(IU/g)

CPK_N – CPK activity in a homogenous section of normal myocardium (IU/g)

CPK_I – CPK activity in a homogenous section of infarcted myocardium (IU/g)

$$K = \frac{DV}{P_{CPK}(CPK_N - CPK_I)}$$

4 IS – infarct size (CPK-g-eq)

BW – body weight (kg)

$$IS = (K)(BW)(CPK_T)$$

5 Given N observed values of CPK activity $E(t_i)$, i = 1,2 ... N CPK_T can be estimated from (see above)

$$CPK_T \approx \sum_{i=1}^{N-1} \bar{f}_i \Delta t_i =$$

$$\sum_{i=1}^{N-1}\left(\frac{\Delta E_i}{\Delta t_i} + k_d \bar{E}_i\right)\Delta t_i = E(t_N) + k_d \sum_{i=1}^{N-1} \bar{E}_i \Delta t_i$$

where

$$\Delta t_i = t_{i+1} - t_i$$

$$\Delta E_i = E(t_{i+1}) - E(t_i)$$

$$\bar{E}_i = \frac{E(t_{i+1}) + E(t_i)}{2}$$

and

$$\bar{f}_i = \frac{f(t_{i+1}) + f(t_i)}{2}$$

Note that $E(t_1) = 0$ is assumed.

Values currently used for constants follow:

Infarct Size Based on MB CPK

k_d (MB CPK) – obtained from serum curve

DV[1]	= 44 ml/kg
$P_{MB\ CPK}$[5]	= 15
MB CPK_N	= 96 IU/g[2]
MB CPK_I	= 25 IU/g[3]
K	= 4 1 x 10

Infarct Size Based on Total CPK

k_d (CPK) – obtained from serum curve

DV	= 44 ml/kg
P_{CPK}[5]	= 15
CPK_N	= 680 IU/g[2]
CPK_I	= 180 IU/g[4]
K	= 5 9 x 10

1 Estimated as plasma volume for man (Nachman H M, James G W III, Moore J W, Evans E I: Comparative study of red cell volumes in human subjects with radioactive phosphorous tagged red cells and T 1824 dye. J Clin. Invest 29: 258 1950)

2 Measured directly as described in text

3 Calculated as 14 % of the estimated total amount of CPK remaining in a region of infarction since MB = 14 % total CPK in normal myocardium

4 Calculated by analogy from percent of myocardial CPK depleted measured directly in conscious dogs 48 hours after coronary occlusion

5 Calculated by analogy based on the empirical relation between CPK released calculated from serum changes and myocardial CPK depletion measured directly in conscious dogs with coronary occlusion.

ACKNOWLEDGEMENT

Ms Susan Wallach's preparation of the manuscript is appreciated.

DISCUSSION

Dr Werkö

Thank you very much Dr Sobel I think many of these topics coming up should be discussed after the next paper but are there any specific questions?

Dr Hjalmarson

I believe that propranolol will not change the CPK disappearance curve when you just study the drug and so will not Arfonad. If you compare the two curves I have the impression that when you change the slope of that CPK disappearance curve you seem to have a larger effect by propranolol compared to Arfonad. Is that your impression too? And one thing more I would like to know how hypertensive these patients are It is obvious that, if you have a real hypertensive reaction, when the patient shows up in the coronary care a reduction in afterload is most important. Are there really hypertensive reactions when you have the result in survival that was a reduction from seven deaths out of ten compared to two out ten I think that is beautiful and I congratulate to the results.

Dr Sobel

Thank you for your comments With such a small series the reduction in mortality should not be emphasized despite the statistically significant result. Furthermore the mortality figures refer only to mortality within one month You posed two important questions one about propranolol and Arfonad. The results with propranolol that were illustrated were obtained in a dog study Our experience with beta-blockade in humans is too limited to merit comment at this juncture and it would be potentially misleading to try to compare the relative amount of salvage with the two drugs until a larger number of patients have been evaluated. You implied something however that I would like to comment upon It has been reported by the group from Montreal that propranolol alters the disappearance rate of CPK. However in our studies with conscious dogs we saw no demonstrable effect of propranolol on the CPK disappearance rate nor did we see an effect secondary to Arfonad If propranolol slowed CPK disappearance which is what has been reported by others our results would actually indicate even more striking salvage of myocardium after administration of this drug than the amount of salvage we estimated.

Dr Mueller

What are your clinical end points using vasodilators in the patient with complicated acute myocardial infarction? In general we consider a systolic arterial blood pressure below 100 mm Hg and a pulmonary artery wedge pressure below 14 mm Hg as contraindication for the use of vasodilators since fall in coronary perfusion pressure and stroke volume could unfavorably influence myocardial oxygenation.

Dr Sobel

In the original series the patients had an average systolic pressure of approximately 160 mm Hg Thus they were not profoundly hypertensive as a group although a few were quite hypertensive on admission The reason we selected hypertensive patients was because a few years ago we were all concerned about lowering the blood pressure in any patient with myocardial infarction and felt that this procedure would be safest and most justifiable in hypertensive patients It has rapidly become common for physicians to endorse vasodilator therapy for many patients with infarction even those with normal or modestly low systolic blood pressure The specific choice of vasodilator may be important A drug such as nitroprusside may lead to rather dramatic reflex tachycardia With Arfonad this occurs much less frequently because the block is at the ganglionic level rather than at the target organ. The level of pulmonary artery wedge pressure is one factor that determines whether or not reflex tachycardia occurs Thus the wedge pressure should be maintained in the range of 12 to 15 mm Hg in patients treated with these agents. Treatment can be safely continued as long as systolic pressure remains above 100 diastolic pressure is not permitted to plummet and cardiac output is maintained or increased Accordingly we are prepared to treat any patient with a systolic pressure exceeding 100, with a high pulmonary wedge pressure as long as the wedge pressure does not fall precipitously or the systemic arterial diastolic blood pressure does not decline dramatically with vasodilator therapy

Dr Just

Dr Sobel your deductions have been very convincing for an infarct which occurs suddenly What would you expect if an infarct develops over a longer period of time or evolves from a preinfarction syndrome or an episode of ischemic pain of longer duration. How would you expect your disappearance curve to be altered and what did you find clinically in such situations?

Dr Sobel

Most of the patients who have what is often called preinfarction angina do not develop enzyme elevations at all Virtually none develop MB CPK elevations. We do not feel that these patients exhibit infarction despite the repetitive pain and occasional electrocardiographic ST-elevation. Because enzyme activity does not increase there is no CPK disappearance curve at all If the patient has a stuttering infarct with repetitive small bouts of necrosis the CPK curve exhibits repetitive small peaks and serum CPK values fail to return to normal for prolonged intervals The method of analysis we use will gi e the same results whether or not enzyme is released very slowly or very rapidly from the heart since the mathematical model takes into account the rate of release However if another parameter changed such as the proportion of CPK released into blood compared to that depleted from myocardium or the fractional disappearance rate from the circulation of CPK (k_d) results would be altered We have no evidence that values of these parameters change substantially in most experimental models or in patients.

THE USE OF HYALURONIDASE AND HYDROCORTISONE IN THE REDUCTION OF MYOCARDIAL INFARCT SIZE FOLLOWING CORONARY OCCLUSION

Experimental and Clinical Observations

Eugene Braunwald, M D and Peter R. Maroko, M. D

One approach to reducing the size of myocardial infarctions has been the administration of hydrocortisone (1) It was observed in anesthetized open-chest dogs with occlusion of the left anterior coronary artery (2) that in pharmacological doses hydrocortisone 50 mg/kg body weight administered 30 minutes after occlusion and 25 mg/kg body weight 12 hours thereafter substantially reduced infarct size as reflected by both myocardial creatine phosphokinase activity (Fig. 1) and histologic appearance 24 hours later (Fig. 2) and also acutely decreased the extent and severity of ischemic injury reflected in ST segment elevations The mechanisms of its protective effect are not clear but several possibilities can be considered. First corticosteroids exert a protective effect on the lysosomes (2) these organelles which are disrupted by acidotic conditions in hypoxic cells, release acidic hydrolases that may contribute to the early irreversibility of cellular damage. There is evidence that a significant proportion of myocardial lysosomal hydrolytic activity shifts from the particulate to the unbound tissue fractions within the first few hours of ischemia (3 4). If these data are applicable to our experimental model then the protective effect of hydrocortisone administered 30 minutes after occlusion could be explained by decreased autolysis Second a stabilization of the phagocytic vacuoles of infiltrating inflammatory cells might also help to explain its action.

Although the effect of corticosteroids in patients with acute myocardial infarction is the subject of controversy (5 6) our findings might help to explain the possible reduction in mortality in patients with acute myocardial infarction treated with hydrocortisone (5)

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EFFECT OF HYALURONIDASE ON INFARCT SIZE

Since hyaluronidase increases diffusion through the extracellular space and may thereby facilitate delivery of substrates to ischemic cells, its influence on the size of experimentally produced infarcts was also analyzed (7). It was found that both the extent and magnitude of ST segment elevations after the

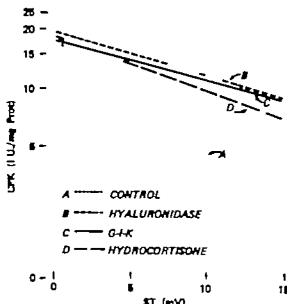


Fig. 1 Relationship between ST segment elevation 15 minutes after occlusion and log creatine phosphokinase (CPK) activity from the same specimens obtained 24 hours later Line A control group (occlusion alone) Fifteen dogs, 101 biopsies. Line B hyaluronidase. Thirteen dogs, 94 biopsies. Line C glucose-insulin-potassium (G-I K) infusion 30 minutes after occlusion, i.e. 15 minutes after ECG recording Thirteen dogs, 96 biopsies. Line D hydrocortisone administration 30 minutes after occlusion. Seven dogs, 42 biopsies. There is a statistical difference ($p < 0.01$) between the slope of line A and the slope of the other lines showing less creatine phosphokinase depression after treatment. (Reproduced by permission from Annals of Internal Medicine 79 724 1973)

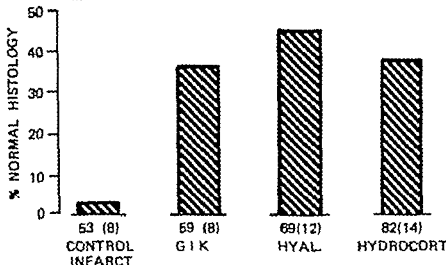


Fig. 2. Comparison of the effect of treatment on myocardial histology in areas with segment elevations over mV First column: control group Second column: glucose-insulin-potassium (G-I-K) group Third column hyaluronidase group Fourth column: hydrocortisone group The numbers below each bar indicate the number of sites biopsied and the number of dogs in parenthesis Note that in all three treatment groups mor than one-third of sites that were expected to show early signs of myocardial infarction were spared. (Reproduced by permission from Annals of Internal Medicine 79 736, 1973)

administration of this enzyme were considerably reduced In related experiments hyaluronidase when administered one-half hour after coronary artery occlusion decreased the depression of creatine phosphokinase activity predicted on the basis of ST segment elevation and also reduced the size of the infarct as evaluated histologically (Fig. 1 and 3) In 45 % of biopsies histologic examinations expected to show early signs of myocardial infarction were normal as a consequence of the administration of hyaluronidase Therefore this enzyme clearly protects the ischemic myocardium f om evolving to an irreversible phase of injury and subsequent necrosis

The administration of hyaluronidase to patients offers several potential advantages compared with other intervention which reduce infarct size after experimental coronary occlusion 1) Its application is simple and does not requ e any special equipment as does intra-aortic balloon counterpulsation) It does not depres cardiac contractility or cause hypotension a does propranolol 3) It does not have the ntrinsic property of changing ST segments as does glucose-insul n-potassium and thus the electrocardiographic monitoring of schemic injury may be used for monitoring the extent and severity of ischemic injury 4) Most importantly hyaluronidase has been used widely clinically and its toxicity is extremely low (8 9) Allergic reactions are rare (0.85 %) generally occurring only after frequent exposure and may be avoided completely if a skin test is performed (10) Finally in terms of effectiveness n educing myoca dial necrosis in the dog after co onary occlu ion hyaluronidase compared favorably with other interventions such as propranolol hypertonic glucose and glucose-insulin-potassium

The precise mode of action of this enzyme in reducing infarct size is not known However it has been suggested (7) that its action is based on its ability to depolymerize hyaluronic acid (11 1?) to increase capillary permeability (13) and thereby to facilitate the transport of energy-producing substances from the blood stream through the interstitium to the myocardial cells Using histologic techniques for staining for hyaluronic acid (Alcian green and Hale s colloidal iron) it was observed that 24 hours after occlusion the quantity of positively stained material in the interstitial space in the center of the infarct was clearly reduced by the administration of hyaluronidase This observation is consistent with the hypothesis that hyaluronidase acts through its depolymeriz ing capabilities and demonstrates that under the conditions of these experiments hyaluronidase reaches the center of distribution of an occluded coronary artery This action could be significant in the presence of coronary occlusion when nutrients must be transported through longer extravascular pathways than when the coronary arteries are patent

CLINICAL OBSERVATIONS

Desp te thei usefulness in demonstrating alteration in the size of an ischemic zone in experimental situation the epicardial mapping technique as well s the technique of sampling for myocardial cre atine phosphokinases and histology are obviously not directly applicable to patients with myocardial

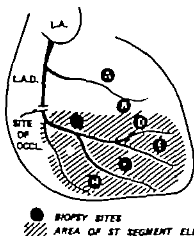
ischemia, although the epicardial ST segment technique can be applied to patients undergoing thoracotomy. Therefore, an attempt was made to develop a method that would be noninvasive and capable of evaluating changes in the size of the injured region. The precordial mapping method was developed in an attempt to determine whether changes in precordial ST segments could be used in a manner analogous to those in epicardial ST segments to assess the severity and extent of the ischemic injury after coronary occlusion (2). Using a multiple lead precordial system in the dog, the effects of various pharmacological and hemodynamic methods were studied. Isoproterenol and arterial hypotension each increased the extent and magnitude of ST segment elevation in precordial as they did in epicardial leads. These observations extended the information previously obtained in open-chest dogs and confirmed the principle that the extent and magnitude of ischemic injury changes after coronary occlusion reflect the balance between myocardial oxygen availability and demand for at least 6 hours after coronary occlusion. Subsequently this method was applied to patients with acute myocardial infarction, using a 35-lead precordial map which permitted the study of changes in ST segment elevation in anterior, anterolateral, or high lateral infarctions.

In view of the apparent lack of toxicity of hyaluronidase and the impressive experimental results with this agent, a pilot study was undertaken to examine its effectiveness in patients with acute myocardial infarction (14).

Twenty-four patients who had suffered typical transmural myocardial infarctions determined by history, enzyme changes and electrocardiographic criteria were studied. The 11 patients who did not receive hyaluronidase served as controls and the 13 patients who received the drug constituted the experimental group. Although these patients were not assigned to one of the two groups in a randomized manner and the design of the study was not blind, there was no attempt to pre-select the patients on the basis of the severity of complication of their disease. All patients had acute myocardial infarction involving the anterior or lateral walls of the left ventricle, and the onset of the chest pain occurred less than 8 hours before the beginning of the study. Patients more than 75 years of age and others with disease of kidney or liver, pregnancy, neoplasms, or infections were excluded.

The control group consisted of seven men and four women averaging 56 ± 4 years (SEM) in age who entered the study an average of 4.7 ± 0.5 hours after the onset of chest pain. The experimental group consisted of 12 men and 2 women, averaging 50 ± 2 years in age, and they entered the study an average of 4.3 ± 0.5 hours after the onset of chest pain. After obtaining informed consent, an intradermal test dose of 150 units of hyaluronidase was given. Each patient then received hyaluronidase 500 National Formulary units/kg body weight intravenously in a bolus, followed by additional identical doses at and 6 hours, and then every 6 hours until 42 hours after the initial dose.

The precordial electrocardiograms were recorded with 35 unipolar leads, as described previously (2). The precordial leads were standard chest electrodes



SITE	ST (mv)	CPK IU/mg prot	HISTOLOGY
A	0	24.6	NORMAL
B	0	18.6	NORMAL
C	3	23.2	NORMAL
D	4	13.4	NORMAL
E	7	7.6	ABNORMAL
F	9	11.2	ABNORMAL
G	8	17.0	NORMAL
H	7	18.6	NORMAL

Fig. 3. The effect of hyaluronidase on the relationship between ST segment elevation (prior to drug administration) and creatine phosphokinase (CPK) activity and histological structure 24 hours later. Left, schematic representation of the anterior surface of the heart and its arteries. L.A. = left atrial appendage; L.A.D. = left anterior descending coronary artery. Shaded area = area of ST segment elevation 15 minutes after coronary occlusion (prior to hyaluronidase administration). Closed circles = biopsy sites. Right, comparison between ST segment elevation 15 minutes after occlusion (before hyaluronidase administration) and creatine phosphokinase activity and histological structure 24 hours later. (Reproduced by permission of the American Heart Association, Inc. Circulation 46: 432, 1972).

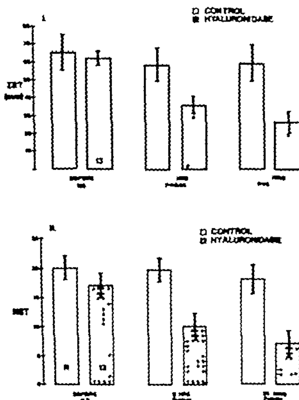


Fig. 4 Panel I The sum of ST segment elevations (ΣST) in control patients and in hyaluronidase-treated patients at zero time (before treatment) and at 2 and 24 hours after treatment. Note that before treatment both groups had similar values of ΣST. However in the treated group ΣST dropped significantly more rapidly than in the control group. Panel II Number of electrodes showing ST segment elevation xceeding 1 mm (NST) in control patient and in hyaluronidase-treated patients at zero times (before treatment), and at 2 and 24 hours after treatment. Note that before treatment both groups had similar alues of NST. However in the treated group NST dropped significantly more rapidly than in the control group. (Reproduced by permission from Annals of Internal Medicine 82 517 1975)

(Hewlett Packard No 14058) n a fixed position in a blanket co ering th precordium distributed in five rows of seven electrodes each

Average ΣST and NST levels before hyaluronidase dministration in this group were not statistically different from the control group. However at all times after treatment with hyaluronidase average ΣST and NST were significantly lower ($p < 0.05$) than in the cont ol group (F g. 5). One patient in the treated group died 10 days after hospitalization due to ventricular arrhythmias. At postmortem examination there wa no sign from gross or microscopic examination of thinning of the ventricular wall or any abnormality of healing of the infarction. Two patient showed signs of ventricular failure one of them overt pulmonary edema both survived. No toxic or allergic side effects were observed in any of the patients who received hyaluronidase

This study showed that the reduction in the magnitude and extent of ST segment elevations was greater in the hyaluronidase-treated than in the control group at each time interval during the 24 hours after treatment. This more rapid decline in the electrocardiographic indices of injury was already evident 2 hours after drug administration. These results support previous observations that hyaluronidase lowers ST segment elevations in the 12 lead electrocardiogram in patients with acute myocardial infarction (15 17). Also although several patients in the non-hyaluronidase-treated control group exhibited an increase in ST segment elevation on sequential electrocardiograms suggesting an extension of the infarction this situation did not occur in any of the hyaluronidase-treated patients. Therefore based on studies in experimentally produced coronary occlusion it is suggested that this reduction in acute myocardial ischemic injury produced by hyaluronidase may reflect a reduction in the quantity of myocardium that eventually becomes necrotic

In a subsequent study carried out on a separate group of 56 patients with acute myocardial infarction the effects of a similar regimen of hyaluronidase administration on the development of Q waves in 35 precordial electrocardiographic leads was examined (18). Thirty-three patients served as controls and 23 received hyaluronidase. Again the times from the onset of pain to entry into the study were similar. At this time the control patients had a total of 438 precordial sites with persistent R waves and with ST segment elevations equal to or exceeding 1.5 mm. These sites were considered to be highly vulnerable for the development of electrocardiographic signs of necrosis i.e. pathologic Q waves and in fact 56 ± 6 % of them did so within one week. In contrast in the hyaluronidase-treated patients a significantly lower ($p < 0.01$) percentage 35 ± 7 % of 294 such highly vulnerable sites developed new pathologic Q waves in the same time interval. The sum of precordial R waves (ΣR) in these highly vulnerable areas declined by an average of 76 ± 4 % (SEM) from an average of 71.5 mm in the control patients and by significantly less, i.e. 53 ± 8 % ($p < 0.01$) from an average of 57 1 mm in the hyaluronidase-treated patients. At the time of entry the control patients also had 487 precordial sites without pathologic Q waves and with ST segment elevations less than 1.5 mm pathologic Q waves developed within one week in 25 ± 4 % of these sites in the hyaluronidase-treated patients

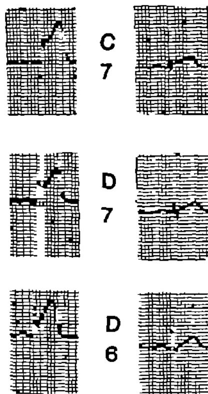


Fig. 5 Three enlarged leads from Fig showing ST segment elevations in these leads before hyaluronidase administration (left) and the striking reduction in ST segment elevation 2 hours after its administration (right). (Reproduced by permission from Annals of Internal Medicine 82: 519 1975).

this percentage was again significantly reduced to 11 ± 3 % of 205 sites ($p < 0.025$)

CONCLUSION

When considered together the observations on the effects of hyaluronidase on the extent of myocardial necrosis in dogs with experimentally produced coronary occlusion (7) the pilot clinical trials demonstrating the effects on the rate of resolution of abnormally elevated precordial ST segments (14), and the development of electrocardiographic changes indicative of necrosis in the QRS complex (18) all suggest that this agent may be effective in reducing the quantity of myocardium that eventually becomes necrotic following coronary occlusion. The lack of toxicity of hyaluronidase and its ease of administration suggest that expanded and rigorous clinical trials should now be undertaken

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DISCUSSION

Dr Hjalmarson

Again I want to congratulate you and your group I will comment upon your earlier discussion this morning, and I am glad you have been cruising on the 35 000 feet to look for the great areas If you had not done that we should not have been here today so I think that you have made a real contribution to this field of cardiology In the data you showed there are of course a number of questions I was very impressed by the fact that maybe mapping is not necessary at all I think that maybe if we take three chest leads and multiply by 10 – I mean by time – you get thirty and you can measure them instead of having forty at just one time I think you get quite a good number both ways The last thing you showed was the development of Q-waves in the ECG and that might be the easiest way to see if you do anything to prevent the heart

One other thing is, how fast will hyaluronidase start its action and how long will the duration be? Are there any side-effects of it?

Dr Bra nwald

Thank you for your questions and your kind comments I am not sure what the proper number of surface leads is and I think that it might well turn out to be less than 35 I am sure you are right. You can begin by saying that for years we have been taking 6 precordial leads and why go any further I think that maybe the thing to do is to take 35 leads and then zero in on those and choose your abnormal sites from those 35 You see if you choose three of these at random you may not find the damaged area, and if you take the 6 classical Wilson precordial leads your electrodes may be too low to detect significant areas of damage You know Drs Shillingford and Thomas have drawn an envelope around the abnormal leads and they have seen that the envelope extends with certain interventions. They of course use 7? leads I think that when we have more experience – then we might be able to say that there are certain leads that have never given us any information, and then we ought to discard those so I think that your point is well taken

I also am quietly elated at the potential of simply looking at the QRS because our final objective is, as I stated at the outset the salvage of contractile myocardium Many good studies have been published in the last 5 years that have shown that patients who suffered myocardial infarction had an excellent correlation between the development of Q-waves and the loss of contractile tissue as observed by localized wall motion disorders on left ventricular angiocardiography there are even now some studies on the extent of the Q-wave and the decline of the ejection fraction in patients.

As far as the hyaluronidase is concerned we know embarrassingly little about it, but we do know that it is essentially devoid of side-effects. It has been used for years in other branches of clinical medicine predominantly for hypodermolysis in pediatrics it has been used by ophthalmologists, and it has no detectable hemodynamic effects. Rarely it can produce sensitization. It is a protein which is extracted from bull testicle We do a skin test and make sure that patients are not allergic. Another thing is that we do not yet know but what concerns us is how sensitization might affect the re-treatment of patients This is something that we hope to find out very quickly now since it looks like we are dealing with a potent agent

Dr Conway

You started your talk by referring to the objective of maintaining a functional myocardium I should like to ask therefore whether you have been able to detect any functional changes say in cardiac output wedge pressure PEP that accompany the improvement in the electrocardiographic abnormalities.

Dr B au wald

Unfortunately we have not done this

Dr Bruyneel

I have a minor question to Dr Braunwald and also to the audience and this is what is the real value of epicardial ST-segment elevation in acute myocardial infarction I think we are a little bit over-estimating the importance of precordial ST-segment results First I would like to give a crude example By looking to an iceberg it is not by pushing it downwards or upwards that we are

changing the size of the iceberg. We have seen this morning many agents increasing and decreasing ST-segment, epicardial or precordial during the very first minutes of acute myocardial infarction. Now I do not know what really is the meaning, because you do not need a transmural infarct to have very high ST-elevation, precordial or epicardial. I know the papers of course from 1972 of Maroko and the statement that epicardial ST segment is an index of severity of an infarct Now the whole question is what you mean with severity of an infarct. As I have read from his papers I think it is a metabolic severe infarct and histological severe infarct. You have not yet correlated with electrical severe infarct. They were not correlated with hemodynamic severe infarcts Those are the two severity parameters we have in the coronary care unit, and I wonder what ST-segment in acute myocardial infarction means in these two situations

Dr Braunwald

The abnormal ST-segment indicates that the myocardium sampled by the electrode whether it is a precordial electrode whether it is an electrode on a single cell or whether it is an electrode placed on the epicardium shows a distinct abnormality of repolarization What does this abnormality correlate with? It turns out that it correlates exquisitely with whether or not the tissue beneath the cell is going to become necrotic a day later and in some studies that Dr Maroko carried out a week later That is an important thing to know What we are looking at at the present time is how well it correlates with the long-term function of that tissue That is a separate question. It is also a very important one and that is why we consider the ST segment to be useful though extremely indirect, index And if an area which previously did not show this abnormality now becomes abnormal, then those cells are without any question jeopardized, and if that abnormality continues the odds are enormous that the cells beneath that lectrode will be necrotic as measured by light microscopy as measured by histochemistry as measured by electro-microscopy and as measured in our first studies with Dr Sobel by means of CPK-concentration.

Dr Bruyneel

Of course we are all interested in ST-mapping because it i so ery easy to do and because it is so very easy to do do you not think we should all obtain the same results Clinically there are some conflicting report – people who did precordial mapping and who did not find any correlation between the improvement of the patient and decrease of epicardial or precordial ST segment in patients in the coronary care unit.

Dr Braunwald

The improvement is measured in what manner

Dr Bruyneel

Well in patients who were in pump failure and there is no direct correlation between ST-mapping results and clinical evaluation

Dr Braunwald

That does not surprise me at all. I would be surprised if there were because one is a mechanical function When you say that there is a discrepancy I think that we have to be sure that we are all measuring the same thing and then that we are also looking at the same outcomes.

Dr Bruyneel

Then, should we consider epicardial and precordial ST-segment just as a qualitative not a quantitative parameter and only for electrical and very indirect for hemodynamical improvements or mechanical improvements?

Dr Braunwald

Well I think it is very dangerous to transfer the ST segments to mechanical function of the ventricle I think that is claim that I do not recall that anyone else has made I would be very surprised if abnormal ST-segments correlate with left ventricular failure Dr Vatner together with Dr Maroko carried out some interesting studies recently In which they have seen ultrasonic gauges to an area and have recorded both ST-segments and mechanical functions from that area The correlation is not good. It is possible for example for a delay to occur in the return of mechanical function following reperfusion, even though the repolarization (ST-segment) abnormality may disappear So all functions of the cell do not come back at once One final point. I think that it is important to point out that the ST-segment can be altered by many processes other than myocardial ischemia. For example the development of pericarditis and serious changes in the patients electrolyte status com-

pletely invalidates this approach It would not invalidate analysis of the QRS complex.

Dr Bruyneel

This is our experience on long-term baboons on 6 hours infarction that ST-segment is a parabolic function of time going up till the 60th minute and there we have the greatest ST-elevation and it comes down gradually and at 6 hours it has come down to 1/3 of the maximum. But at that time by re-opening the chest you still have very severe infarct but you also have fibrin around and pericarditis

Dr Braunwald

You can also get changes in the relationship between the electrode and the tissue If that particular portion of the myocardium has been damaged by the electrode itself Then you are no longer getting a representative sample and spurious ST segment changes may occur

Dr Werkö

I think we have to conclude this part of the session and thank you very much again Dr Braunwald.

PROPRANOLOL IN ACUTE MYOCARDIAL INFARCTION IN MAN EFFECTS OF HEMODYNAMICS AND MYOCARDIAL OXYGENATION

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Clinical and experimental observations, made during the past decade, support the belief that myocardial infarction develops in a stepwise manner and that myocardial tissue may be salvaged by techniques designed to interrupt this progressive necrotic process (1-4) Beta adrenergic blockade (5 7), peripheral vasodilatation (8-11) cardiac assistance (12 18) and early coronary artery surgery (19-21) have all been proposed as therapeutic interventions which might salvage myocardial tissue and decrease infarct size. Our early observations that beta adrenergic stimulation produced metabolic deterioration in different stages of coronary artery disease (14 22), together with parallel studies revealing the frequent occurrence of augmented myocardial free fatty acid uptake in complicated myocardial infarction (23) suggested that beta adrenergic blockade might be useful in limiting myocardial injury

Twenty patients, 19 men and one woman were studied within 12 hours after the clinical onset of myocardial infarction. The criteria for acute transmural myocardial infarction were (1) development of Q wa es and presence of ST segment elevation in the electrocardiogram (2) enzyme elevations and (3) history characteristic for myocardial ischemia. The average age of the patients was 55 years and ranged from 36 to 74 years The electrocardiogram revealed anterior wall infarction in five anterior-lateral wall infarction in eight and inferior wall infarction in four In three instances the acute inferior wall infarction was associated with severe anterior-lateral wall subendocardial ischemia. Peak creatine phosphokinase averaged 1065 IU/L (normal values 0-80 IU/L) initial arterial free fatty acid concentrations 987 μM/L, and arterial lactate and glucose contents 1 7 mM/L and 184 mg/100 ml respectively Patients were not included in the study if one or more of the following findings were observed. (1) symptoms of cardiac failure such as cardiac enlargement dyspnea or bibasilar rales (2) pulmonary venous congestion on radiography (3) systolic arterial cuff pressure of less than 100 mm Hg, (4) heart rate below 65 beats/min (5) atrioventricular or intraventricular conduction delay (6) history of chronic lung disease and asthma, (7) diabetes mellitus.

Catheters were placed into the pulmonary artery the coronary sinus and the brachial artery Cardiac output was determined by the direct Fick method coronary blood flow was measured by a modification of the method of Krasnow using I[125] antipyrine as the indicator (24). Details about techniques determinations of blood concentration of oxygen, lactate glucose of blood pH oxygen and carbon dioxide tensions were described previously (25) Free fatty acids in serum were determined according to the method of Dole (26) and Trout (27) 1-norepinephrine in serum was measured using the trihydroxyindole method (28) Propranolol 0 1 mg/Kg body weight, was administered intravenously within 10 minutes in 3 divided doses. Hemodynamic and metabolic measurements were taken before and 15 minutes after the last dosage of propranolol.

Fig. 1 shows that heart rate decreased following propranolol in all but three patients the mean changed from 80 to 73 beats/min. This relatively small change in heart rate may be related to the fact that heart rate was below 80 beats/min in 50 % of on patients probably due to increased vagus tone during the acute phase of infarction particularly in inferior wall infarction All measurements of arterial pressures decreased following propranolol mean arterial pressure from an average of 92 to 76 mm Hg. Time-tension index, the product of systolic mean pressure and heart rate uniformly decreased The most important hemodynamic response to propranolol in our patients appeared to be a substantial decrease in myocardial contractility This

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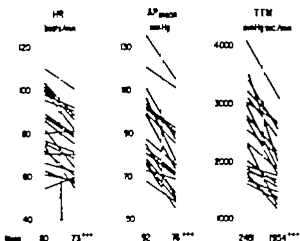


Fig. 1 Hemodynamic effects of propranolol in individual patients. Control values are shown at the left, result after propranolol at the right of the horizontal axis. Heart rate (HR), mean arterial pressure (AP_{mean}) and time-tension inde per minute (TTM) uniformly decreased after propranolol.
p <0 001 p <0.01 p <0 05

decrease was reflected by a fall in cardiac output (Fig. 2) and arterial pressure with little changes in systemic vascular resistance. The fall in cardiac output was mainly due to a fall in stroke volume The decrease in myocardial contractility was further reflected by a decrease in myocardial oxygen consumption per beat (Fig. 2) This change is mainly related to diminished tension development of the myocardium since heart rate is eliminated as a determinant of myocardial oxygen requirements The response of the pulmonary artery wedge pressure to propranolol was initially surprising (Fig. 2) Wedge pressure decreased in the patients with the highest value prior to propranolol, suggesting that changes in left entricular compliance rather than in left entricular volume played an important role in these results Evidence is accumulating in clinical and experimental studies that acute ischemia decreases the compliance of the myocardium. Scheldt *et al* observed in patients with acute angina pectoris sharp elevations of left ventricular end diastolic pressures prior to changes in heart rate and blood pressure (29) Direct volume measurements revealed that end diastolic volume was unchanged during pacing induced angina pectoris (30). Mc Laurin *et al* demonstrated a decrease in left ventricular peak negative dp/dt during acute ischemia, indicating impaired diastolic relaxation (31). Our observations that propranolol decreased or produced but little changes in pulmonary artery wedge pressure while myocardial contractility decreased might be interpreted in light of compliance changes discussed above Similar observations of improved compliance of ischemic myocardium by beta adrenergic blockade were reported by other investigators (32, 33)

Propranolol decreased coronary blood flow in all but one patient averaging 77 and 64 ml/100 g/min before and after treatment respectively (Fig. 3). Coronary vascular resistance increased in most instances Although our measurements of total coronary blood flow do not provide information relative to distribution of blood flow within the myocardium experimental studies demonstrated that propranolol did not decrease blood flow in ischemic areas (3., 33) but did improve perfusion of subendocardial regions of the ischemic zone (34) Myocardial oxygen consumption decreased following propranolol an average of ? ml/100 g/min the results were rather parallel to those of coronary blood flow (Fig. 3).

Propranolol induced changes in myocardial perfusion and oxygenation depend upon the effect of beta adrenergic blockade on the determinants of myocardial oxygen consumption heart rate myocardial contractility and wall tension. Decreasing contractility heart rate and diastolic tension development (in ischemic myocardium) decrease oxygen consumption. Opposing this are propranolol induced increases in ventricular volume and fiber length increasing wall tension and oxygen consumption. Our studies demonstrating a 22 % decrease in myocardial oxygen consumption per minute and a 1 % decrease in oxygen consumption per beat indicate that decreases in contractility and

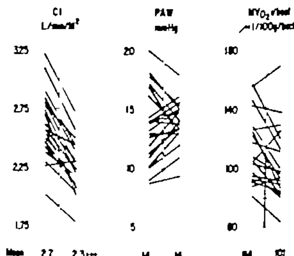


Fig. 2. Hemodynamic effects of propranolol in the individual patients Control values are shown at the left results after propranolol at the right of the horizontal axis. Cardiac index (CI) and myocardial oxygen consumption per beat (MVO_2/beat) decreased after propranolol. Pulmonary artery wedge pressure (PAW) decreased or produced but little changes.
p <0 001 p <0.01

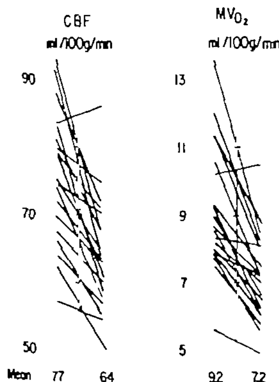


Fig. 3. Effects of propranolol on myocardial perfusion and oxygenation. Control values are shown at the left, results after propranolol at the right of the horizontal axis. Coronary blood flow (CBF) and myocardial oxygen consumption (MVO_2) uniformly decreased after propranolol.
*** $p < 0.001$

heart rate outweighed any increase in myocardial wall tension. Further evidence that these decreases in oxygen consumption represent diminished myocardial oxygen demand is the narrowing of the arterial-coronary sinus oxygen difference and the striking improvement in myocardial lactate metabolism. Fig. 4 shows the two components of myocardial oxygen consumption as coordinates. Decrease in coronary blood flow was associated with a decrease in the arterial-coronary sinus oxygen difference in 15 patients the change for the total patient group averaged 0.71 ml/100 ml ($p < 0.01$). The combination of decrease in coronary blood flow and myocardial oxygen extraction with an increase in coronary vascular resistance is characteristic for coronary autoregulation, emphasizing that propranolol diminished myocardial oxygen requirements. Propranolol markedly improved myocardial lactate metabolism (Fig. 5). All of the five patients who initially produced lactate shifted to lactate extraction from an average of -8 % to 14 %. In the remaining patients propranolol increased the rate of lactate extraction, the average of the entire group increased from 14 % to 25 %.

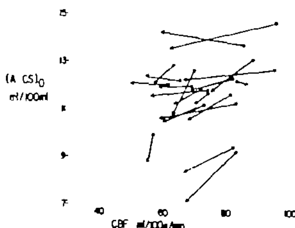


Fig. 4 Effect of propranolol on myocardial oxygen consumption. Propranolol decreased both components of myocardial oxygen consumption, coronary blood flow (CBF) and, in most instances, arterial-coronary sinus oxygen difference $(A\text{-}CS)_{O_2}$.

Beta adrenergic blockade changed substrate utilization of the myocardium as indicated by an increase of the myocardial respiratory quotient from an average of 0.81 to 0.93 ($p < 0.001$) (Fig. 5). Utilization of both substrates glucose and free fatty acids probably was altered. As will be shown later in this paper propranolol remarkably diminished accumulation of lactate and hydrogen ions in ischemic myocardium in animal experiments. Relating these findings to our observations in the pa-

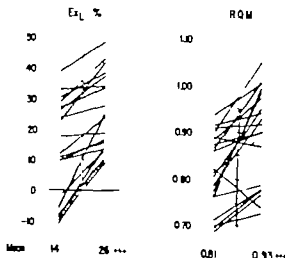


Fig. 5 Effect of propranolol on myocardial metabolism. Control values are shown at the left, results after propranolol at the right of the vertical axis. Myocardial lactate utilization improved. Production of lactate (Ex_L) shifted to extraction or the rate of lactate extraction *increased*. *Propranolol* increased the respiratory quotient of the myocardium (RQM) in the majority of patients.
*** $p < 0.001$.

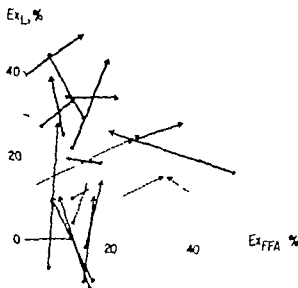


Fig. 6 Interrelationship bet een myocardial lactate (Ex_L) and free fatty acid (Ex_{FF}) extractions. Propranolol produced a shift from lactate production to extraction without change in free fatty acid uptake (narrow dark lines). I four instances with initial low lactate extraction propranolol increased both lactate and free fatty acid uptake (dotted lines).

tient propranolol may have enabled the ischemic myocardium to enhanced glycogen breakdown and glycolysis On the other hand, propranolol probably decreased mobilization and myocardial utilization of free fatty acids. The arterial free fatty acid content decreased following propranolol from an average of 938 to 823 μM/L, although these changes were statistically not significant

The interrelationship between myocardial lactate and free fatty acid extraction is shown in Fig. 6 Free fatty acid extraction was either unchanged or decreased in the five patients who produced lactate prior to propranolol (narrow dark lines) Four of the five patients who had lactate extraction below 1 % (dotted lines) increased myocardial free fatty acid extraction as well as lactate extraction with propranolol

Coronary sinus sampling provides venous effluent from ischemic and non ischemic myocardium In order to be able to tudy more closely ischemic zone metabolism and propranolol induced changes we ligated the descending coronary artery in 10 open chested mongrel dogs and sampled from the great cardiac vein Severe myocardial lactate production after coronary artery ligation a eraging 50 % was shifted to an verage extraction of 15 % by propranolol (Fig. 7) Changes in coronary e nous pH had a similar trend than those of lactate

The sharp drop in pH from an average of 7.37 to 7 26 after ligation reversed to an average of 7.35 following propranolol indicating that beta adrenergic blockade must have improved ischemic zone metabolism The interrelationship between changes in the arterial-coronary venous difference of lactate and pH is demonstrated in Fig. 8. Changes observed after coronary artery ligation (circles) accumulated in the left lower quadrant, indicating lactate production and increased production of hydrogen ions. Changes induced by propranolol clustered in the right upper quadrant, indicating increased myocardial utilization of lactate and less accumulation of hydrogen ions. Changes in lactate utilization and pH were closely related ($r = 0.76$ $p < 0.001$)

The oxygen saving effect of propranolol is predominantly based on depression of cardiac hemodynamics. Its humoral effect however may also be of importance Fig. 9 shows *l*-norepinephrine contents in the arterial and coronary venous blood in experimental myocardial infarction. The l-norepinephrine levels were high at the control state due to manipulations of the open chested dog. After coronary artery ligation the l norepinephrine contents in the coronary venous blood exceeded those in the arterial blood indicating catecholamine release by the ischemic myocardium. Following propranolol these changes were reversed. The coronary venous l-norepinephrine levels were either similar to or lower than the arterial contents. In some instances the myocardium appeared to be able of l-norepinephrine reuptake from the coronary circulation It has been shown in animal experiments that acutely ischemic myocardium releases catecholamines (35-37) mediated by activation

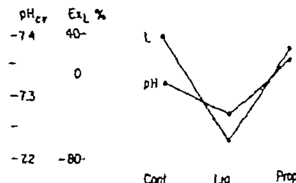


Fig. 7 Propranolol induced changes in myocardial extraction (Ex) of lactate (L) and of coronary venous (CV) pH in e perimental myocardial infarction. Average lactate extraction of 21 % at the control state (Cont.) shifted to production of 50 % after coronary artery ligation (Lig). Propranolol (Prop) reversed these abnormalities. Change in coronary venous pH showed a similar trend. p 0 05

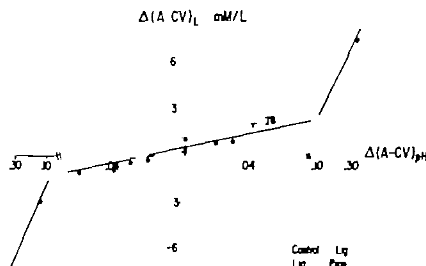


Fig. 8 Interrelationship between changes (Δ) in arterial-coronary venous differences (A-CV) of lactate (L) and pH. Changes, observed after coronary artery ligation (Lig.) accumulated in the left lower quadrant, indicating myocardial lactate production and increased production of hydrogen ions. Propranolol (Prop.) induced changes clustered in the right upper quadrant, indicating increased utilization of lactate and less accumulation of hydrogen ions.

of sympathetic receptors in the myocardium by a decrease of pH in the ischemic area and other mechanisms. Propranolol decreasing the response to adrenergic stimulation and improving the metabolic condition of the myocardium seems to be able to diminish local catecholamine release and thus myocardial oxygen requirements.

All 20 patients tolerated the intravenously administered propranolol well. None developed dyspnea or other clinical findings of left ventricular failure

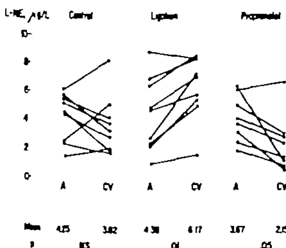


Fig. 9 Arterial (A) and coronary venous (CV) l-norepinephrine (L-NE) contents in experimental myocardial infarction, and effect of propranolol. After coronary ligation, L-NE contents of the coronary vein exceeded that in the artery indicating L-NE release by the ischemic myocardium. Propranolol reversed these changes. I some instances the myocardium appeared to be able of L-NE uptake the coronary venous content was lower than the arterial.

Perfusion of the skin and urine output remained adequate. Angina pectoris unresponsive to meperidine or morphine therapy disappeared in four patients after propranolol. All patients survived and left the hospital. After the pilot study reported in this paper the propranolol regimen was applied to an additional 34 patients in whom chest pain recurred after acute myocardial infarction. Four hours after the initial intravenous administration propranolol was continued orally in general 80-100 mg per day. One of the 34 patients with persistent chest pain developed an inferior wall infarction in the presence of an acute anterior wall infarction, went into cardiogenic shock and died. Two patients with recurrent chest pain while on propranolol therapy extended their infarction, but remained stable. The remaining 31 patients did well.

Our observations in human myocardial infarction demonstrate that beta adrenergic blockade improved oxygenation of ischemic myocardium. Decrease in heart rate and myocardial contractility improvement of ventricular diastolic relaxation and probably decrease in catecholamine release by ischemic myocardium represent the main factors, diminishing myocardial oxygen requirements. Administered early to patients with acute myocardial infarction, propranolol might interrupt the stepwise development of myocardial necrosis salvage potentially viable myocardium and improve both immediate mortality and long-term ventricular function.

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DISCUSSION

Dr Hjalmarson

You had six patients with a high pulmonary wedge pressure and you had six patients with a lactate production and FFA production. I wonder if these might be the same patients.

Dr Mueller

The patients with the high pulmonary artery wedge pressure are not those who show myocardial production of lactate and high uptake of free fatty acids

Dr Braunwald

Do you have any electrocardiographic observations on these patients

Dr Mueller

No we have not

THE EFFECT OF β-BLOCKADE ON ST SEGMENT ELEVATION AFTER ACUTE MYOCARDIAL INFARCTION IN MAN WITH SOME EXPERIMENTAL OBSERVATIONS

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Sussex
England

Patient survival in acute myocardial infarction depends essentially on three main determinants circulation failure cardiac arrhythmias and the prognosis for the myocardial infarction itself Within the last fifteen years considerable progress has been made in the first two and the third is now the centre of interest

Early study of the circulation changes during acute myocardial infarction in apposition with assessment of neurosympathetic drive suggested that tachycardia skin pallor and sweating, and haemodynamic behaviour of the peripheral circulation did not always follow the homeostatic principles thought to compensate for circulation failure (1). Rather there was a varying, and sometimes dramatic increase in neurosympathetic drive (2), sometimes unrelated to circulation events but especially associated with pulmonary congestion and oedema (3).

It was thought possible that adrenergic drive excessive for the requirement to maintain the circulation might have harmful effects Experimental work (4 5) suggested that catecholamines might precipitate arrhythmias in acutely ischaemic hearts and also might damage the myocardium. In respect to patient arrhythmias in the acute illness clinical trial was disappointing in that β-blockade was ineffective in arrhythmia prophylaxis and saving of life (6 7 8, 9). In respect to effects of catecholamines on the myocardium and protection by β-blockade the evidence is as yet incomplete

Raab (10) showed that experimental stimulation of the stellate ganglion led to ST segment elevation when coronary flow was restricted The principle was supported by the experiments of Maroko *et al* (11) who indicated that catecholamines exaggerated ST segment elevation overlying acutely ischaemic myocardium β-blocking agents educed ST segment height. Because of the discrepancy (4 5 6 7 8 9) found between experiment and patients in the case of arrhythmias sociated with catecholamine stimulation, Pelides t al explored the effect of practolol on the ST segment in patients with acute myocardial infarction (12) By 72 lead frontal electrography it was established that β-blockade could reduce the ST segment height in man with acute myocardial infarction The effect was to reduce both the area within which the ST segment was elevated and the degree of elevation The overall reduction in height was about 20 % both at the centre and edge of the area in which ST was elevated

It seemed likely that this effect was due either to a direct effect on the heart and especially on the infarct itself or on the circulation, which might secondarily affect the heart. Haemodynamic studies failed to show major changes in the systemic circulation as regards the mechanical load on the heart (13) and it was clear particularly that the ST segment could be markedly educed without a e duction in heart rate (1) (Fig. 1) A direct effect was responsible

Because of the technical and ethical problems of analysing the mechanisms whereby β-blockade di-

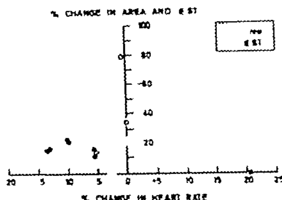


Fig. 1 Changes in heart rate in relation to changes in ST segment elevation and the area of ST segment elevation. N clear elationship between heart rate reduction and reduction in ST segment height and area was found after practolol. (Reproduced by permission from the Editor of Cardiovasc. Res. (1972), Vol. 6, No. 3 295-301).

rectly reduced ST segment elevation in acute myocardial infarction in man further experimental work was undertaken.

One main concept in the evaluation of "ischaemia" is that ischaemia (i e shortage of blood) is relative to the metabolic demands for oxygen (neglecting considerations for anaerobic respiration and the removal of metabolic products) Thus the oxygen delivered to heart muscle must be sufficient to allow aerobic metabolism generating energy for "external" mechanical work and "internal" metabolic overheads When anaerobic metabolism is activated some sparing for oxygen requirement results but this is normally very small in relation to the aerobic contribution for energy production.

Thus oxidative metabolism (plus a small anaerobic contribution) is required for "external" mechanical work plus "internal" metabolic work

In order to quantify oxygen supply preliminary work involved cannulating the great coronary vein in dogs and delivering the coronary venous blood to the right atrium via a bypass A cuff flow meter was placed in series with the bypass. A snare around the anterior descending coronary artery at the same level as the coronary venous cannula (proximal to the junction of the *venae commitantes*) allowed the onset of ischaemia within the myocardium drained by the cannula and great coronary vein system. With total arterial obstruction the venous flow usually fell to between 10 and 30 %. The pattern of flow with time was variable Arteriovenous differences for various metabolic substances showed that there was no major change including lactate discharge after propranolol sufficient to reduce ST segment to a major extent (14) Some uncertainty as to these results followed a consideration of the venous flow factor from which metabolic balance was calculated If propranolol reduced non-ischaemic myocardial flow (as it does) then the less ischaemic tissues (which may also be discharging lactate) may change in terms of (blood flow × A V difference) in a fashion different from the more ischaemic tissues where the flow behaviour after propranolol could not be predicted Furthermore the tissues at the edge of the ischaemic lesion might have a different venous drainage The interpretation of the net flow change in the great coronary vein and derived metabolic balance would be difficult and the interpretation of coronary sinus data in man (which would be an attractive possibility) would also be difficult

It was decided to characterise myocardial blood flow in the experimental ischaemic area with point to point measurement using radioactive microspheres (15) Multiple studies indicated that the ischaemic lesion usually chosen for experimental study has a widely varying myocardial blood flow. In the centre the blood flow was least (0 – 20 % of control) and approaching the edge of the cyanosed patch the blood flow was greater (60 – 80 % of control) Around the lesion outside the cyanosed area a rim of hyperaemia was found. At all points in the cyanosed lesion there was a tendency for endomyocardial blood flow to be less than epicardial flow

In preparations in which ST segment elevation was stable half an hour after the onset of myocardial ischaemia, propranolol was administered intravenously in a dose sufficient to produce marked reduction in the height of the ST segment This dose (0 08 mg/Kg) did not lead to changes in the systemic circulation sufficient to cause confusing secondary effects due to change in mechanical load on the heart Maps of regional myocardial flow including distinction between epicardial and endocardial flow were made before and after propranolol with Sr^{85} and ^{141}Ce labelled microspheres Maps of ST segment height were recorded before and after propranolol It was thus possible to make a point to point comparison of ST segment reduction and local myocardial blood flow

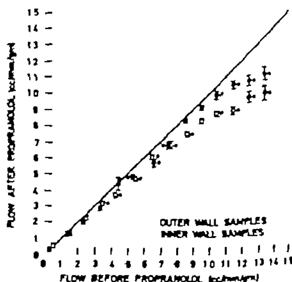


Fig Effect of propranolol on regional left ventricular blood flow Inner and outer wall samples from a series of experiment are grouped horizontally according to the initial flow in increment of 0 1 ml/min/g The mean flow for each range of samples is shown on the horizontal axis and the flow after propranolol on the vertical axis The continuous line is the 45° line of identity The bars indicate one standard error of the mean and the asterisks show that the flow values before and after propranolol are significantly different (p <0 05 paired t-test) Each point represents the mean of at least 11 samples, most contain more than 20 samples and some more than 60 Deviation of point below the identity line indicates reduction in flow after propranolol (Reproduced by permission from the Editor of Cardiovasc Res (1975), 9 No 2, 178-186)

The overall effect of propranolol was to reduce myocardial flow or lead to no change. Where initial blood flow was less than 0.3 ml/min/g⁻¹ propranolol caused small decreases in flow which were not statistically significant where flow exceeded 0.3 ml/min/g⁻¹ propranolol caused a significant flow reduction that was more marked in outer than inner wall samples (Fig. 2).

The effect of propranolol on ST segment height (Fig. 3) was much the same within the cyanosed area. Elevated ST segments became less elevated or isoelectric and isoelectric segments became depressed. Over non-ischaemic areas propranolol caused a small but significant ST depression.

In seeking a mechanism for the means whereby propranolol reduces the ST segment height it is thus possible to say that propranolol does not primarily increase blood flow. Flow remains unchanged or falls. Thus it is not possible to explain reduction in "ischaemia" (equivalent to ST segment height) through a primary improvement in cell oxygenation. What alternative possibilities remain? If the imbalance between oxygen supply and metabolic demand has been improved then propranolol must reduce metabolic demand either by reducing "external" mechanical work or "internal" metabolic overheads.

Does propranolol reduce *external mechanical* work? In these experiments propranolol produced no significant changes in heart rate (although some fall in rate was seen in individual experiments) and in man, it is known that β-blockade can be associated with great reduction in the ST segment without a fall in rate (12). Mean arterial pressure in the experiments fell by less than 2 % and in man the fall in pressure and changes in flow produced by clinical doses of β-blocking agents are quite small in most patients (13). Thus overall heart work reduction is not a prerequisite for ST segment reduction.

What about regional work? The quantification of regional work in an ischaemic area is very difficult. It is likely that tissues with different degrees of oxygenation across the ischaemic area produce differing amounts of tension and shortening. Moreover the ischaemic area is subject to forces generated around it by the non-ischaemic tissue. One thing is sure. The external work is small as compared with normal tissue (16). During heart contraction the ischaemic tissues lengthen. This may be visually observed as a localised systolic expansion both in animal experiments and in man. The expansion may be so obvious as to be palpable through the chest wall in patients. Powerful inotropic agents such as isoprenaline do not seem to improve the contractions of acutely ischaemic areas (18) and may depress them further (19, 20). The effect of propranolol

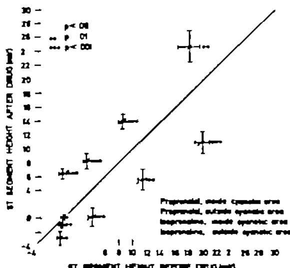


Fig. 3. Effect of propranolol and isoprenaline on ST segment height. Values are grouped horizontally according to the initial ST segment height, means for each group after drug are shown on the vertical axis. The bars represent one standard error of the mean and the asterisks indicate statistical significance of the difference between pre-drug and post-drug means (paired t-test). The continuous line is the 45° line of identity. Each point represents the mean of at least 10 sites and most contain 15-20 values. Propranolol caused a reduction in ST segment height at all initial values. (Reproduced by permission from the Editor of Cardiovasc. Res. (1975), 9, No. 2, 178-186).

on the ST segment (often 80-90 % reduction) therefore seems disproportionate to possible metabolic savings or loss from changes in contraction of the ischaemic region.

It is possible that propranolol reduces "internal" myocardial metabolic work. Catecholamines are reputed to lead to "inefficiency" in terms of generating energy from a given oxygen and substrate supply. An improvement in "efficiency" might follow catecholamine blockade. Here too the changes are likely to be very small at the levels of oxygen consumption (less than 10 % of normal) found in the centre of the ischaemic lesion and it is here that large reductions in ST segment height may be found, e.g. Fig. 4.

Does propranolol (and other β-antagonists) reduce ST segment height by a direct effect on membrane repolarisation? This is possible but not supported by comparable effects on non-ischaemic myocardium. Is the ST response due to very small, but true changes in the state of ischaemia? This is also possible but it would require an obvious alinearity in the relation between ST segment height and the ischaemic state (in view of the very large reduction in ST height without increase in blood flow in tissues which are not contracting).

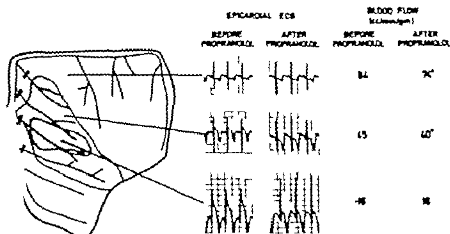


Fig 4 Composite figure showing marked reduction of ST segment height in the centre of a typical experimental ischaemic lesion after propranolol. The corresponding myocardial blood flow values show no change after propranolol.

To what extent are acutely ischaemic tissues made more viable by β-blockade? It seems unlikely that myocardium with less than 10 % blood flow (which is often the case in the centre of myocardial infarcts) can be rendered viable in the long term without an increase in blood flow whatever the ST segment reduction. It is possible that tissues with higher ranges of flow in the periphery of an infarct may be sufficiently improved in their metabolic balance of payments so as to retain their structural integrity. Even here a future contribution in terms of mechanical work pre-supposes that adequate collateral blood supply becomes established. It is therefore logical that studies of the ST segment shift as an index of future cell viability should run parallel with an assessment of the available regional myocardial blood flow.

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DISCUSSION

Dr Muir

The metabolic effect of propranolol probably enhancing myocardial carbohydrate and decreasing free fatty acid utilization, may be of more importance than we realize. Oxidation of glucose is more efficient than that of free fatty acids. The energy obtained from a given amount of oxygen is less for lipids than for carbohydrates

Dr Thomas

Are you saying that beta-blockade lowers the systemic FFA concentration and maybe changes the balance of substrate utilization round to a more "efficient" combination – glucose rather than FFA?

Dr M eller

Our results clearly demonstrate that propranolol increased the respiratory quotient of the myocardium *in patients with acute myocardial infarction* Arterial free fatty acid contents decreased after propranolol although these changes were statistically not significant.

Dr Thomas

One difficulty I have is a quantitative one It really is a considerable shift in ST-segment, is it not? And if one seeks proportionality and linearity in an explanation, something else has got to move considerably too An alternative is alinearity When all data – flow versus ST-segment – were put together there was an approximate relation But point to point wise we found considerable difficulty in drawing a straight line It may be that the relation is not as straight as we think And if it is alinear – then a small change in true "ischemia" or anything else responsible for ST shift – may produce large ST-segment shift.

Dr Maroko

I share your impression that the linearity between ST-segment elevation and other signs of necrosis is lost after certain heights of ST segment elevation probably somewhere between 7 and 10 mV on the epicardium Apparently all sites with ST-segment elevation will develop QS s and will develop total necrosis as we can judge by CPK Therefore if this critical height of ST-segment would already develop to maximum depression in CPK and maximum evolution of QS probably higher ST segment elevations would not be additive since there is already 100 per cent necrosis I do not know what that extra height represents

Dr Thomas

The ST-segment elevation of course i the part of the activation potential which relates to repolarization. It occurs at a membrane level dependent on DC potentials through ionic flux. It is a membrane phenomenon. Is it possible that these agents act specifically at the membrane level? Of course it does not exclude them working through other mechanisms loading versus supply in another sense It is possible that they have several points of attack, and they may not be necessarily closely related. Enzyme loss essentially relates to a loss in function of one sort or another at the membrane level The enzyme molecule has got to get across the membrane and it could be that an essential part of the infarct functional disease is a membrane disease and that is what we are studying through ST-segment shift and enzyme loss. And in that instance it would add up very particularly that beta-blocking agents influence ST-segment shift at the membrane level.

I have screened about a dozen agents for ST segment influence and it does seem to be particularly related to the beta-adrenergic phenomenon Maximum ST-segment shift is very much related to beta-adrenergic activity Agents which have a very distinct local anesthetic quinidine effect, such as d-propranolol particularly do not shift it at a dose many times greater than that required for l-propranolol

Dr Hjalmarson

I think it is important to stress that when you have developed a center of myocardial infarction the area with ST-elevation or reduced coronary flow it is not a measure of ischemia. I think that in the critical center you get a marked release of catecholamines from nerve endings and that will labilize all the membranes and you get a leakage outwards and inwards of ions through the cell membranes. Potassium will leak out and potassium in itself changes other nerve endings in the surroundings that will release catecholamines Even if you have a quite proper coronary flow somewhere out in the periphery you have the central kind of explosion of catecholamines that will spread out. That means that even if you have some coronary flow the diffusion of catecholamines will result in cellular leakage of potassium that will interfere with norepinephrine release We have to remember that what we measure with ST-elevation is the change of ions over the membranes so I think it could be a shift in ions and in volumes and not necessarily a measure of the coronary flow in that area. I am not surprised to find the discrepancies you have between regional coronary flow and the ST-elevation.

Dr Thomas

A quick remark – Timothy Regan in New Jersey showed that potassium infusions into the coronary

artery under these circumstances elevate ST-segment.

Dr Mjøs

I cannot solve your problem but I can tell you that we have got exactly the same problem – if it is a problem – as you with the anti-lipolytic agent clofibrate which does not influence myocardial blood flow to the ischemic zone but nevertheless effects a marked reduction in the ST-segment elevation So it is not a beta-blocking effect *per se* As Dr Mueller pointed out the beneficial effect of both agents may be due to reduced oxygen demand through a shift in the metabolism from free fatty acids to glucose

Dr Thomas

My difficulty is balancing the metabolic bank account. With considerable reduction in ST segment height for instance with propranolol maybe 80 per cent – how can I equate that with metabolic sparing of the same order in tissues that are not mechanically working such as to produce external work?

Dr Braunwald

I think we are lucky if we are OK directionally But to look for an 80 per cent reduction in the ST-segment and to consider that that may be related to an 80 per cent of something else It is conceivable that a change in mitochondrial PO_2 of half a mm Hg might make a difference that could have a profound effect on a whole series of cellular reactions One would not expect an 80 per cent change and that could have a very significant effect on the ST I certainly have never thought that it was linearly related to anything

Dr Thomas

I think we have to face this one out because at the end of the line there has to be a very convincing argument before it is acceptable to use ST-segment shift as a measurement of ischemia – which we both hope we can do And the most obvious one is indeed the argument that you have produced over many years oxygen supply versus metabolic demand.

Dr Braunwald

Yes, but not related in linear relation

Dr Thomas

Might it be extraordinarily alinear?

Dr Maroko

It probably is a critical point For example it is possible to do a very simple experiment to constrict the coronary artery in a dog and measure the epicardial ST and find that there is a reduction of coronary blood flow to approximately 65 per cent and a reduction of myocardial oxygen delivery to about 65 per cent of control before there is any abnormality in the ST-segment

Dr Thomas

If you look at the centre of the lesion the flow is less than 20 per cent, and so in terms of balancing accounts if it is alinear and we do get to a point of inflection in the relation, the absolute profit for that ischemic section with reduction in ST-segment may not be very great in relation to ST-shift.

Dr Braunwald

Well it may not be very great but it is enough to salvage the tissue when you look at it microscopically the next day Now let me go back to the fact that the cells are not working And that is an over simplification the ST is an oversimplification of a complex series of electrical events and that slide you showed is an equal simplification of complex mechanical events It does not mean the beta-blocking agent is not acting on individual cells It is possible for example to show different degrees of bulging which indicates that the beta-blocking agent could still be exerting an oxygen sparing effect at a time when no external work is performed

Dr Thomas

If you look across the lesion an enormous range of absolute blood flow is found Each cell has got its own specific supply-demand problem We really have to know the demand problem right across the lesion. It is at this point that engineers throw their hands in hands in horror in terms of calculating external work, and – even worse – total work But if you look at the ST-segment shift in patients after beta-blockade it is much the same from the centre to the so called edge I cannot easily equate that observation with the range of supply-demand problem that would be very reasonable to foresee right across What I am saying is that something is shifting ST-segment in a fashion not to be equated with

the range of supply and demand problems one would anticipate as a profile across the ischemic lesion.

Dr Braunwald

I cannot help you with that last one. Except to the point that interventions which diminish oxygen needs having nothing to do with adrenergic drives such as unloading by means of counterpulsations exert precisely the same effect. I hope that no effort is being made to call out of this method something that it is intrinsically unable to deliver and that is a high degree of precision. I hope that we do not push this method beyond what it could under the best circumstances offer which is directional change.

Dr Hjalmarson

I would like to remind you that the greatest problem is norepinephrine in the heart and not the circulating catecholamines. We are supposed to have 10 M catecholamines in the circulating blood in a homogenate of a heart we have 10^{-6} M and in the sympathetic nerve ending in the heart 10^{-3} M norepinephrine. If we get a certain degree of ischemia in the myocardium, that will give a steady leakage of norepinephrine to the surrounding tissue. This will in turn release potassium from the cells which will release more catecholamines from other nerve endings. Now if you give a beta-blocker you will immediately switch off the effects of norepinephrine which will be released when you have a high potassium concentration. You can pump potassium into the cells and you can change the membrane potential which means that the membrane function can come back in the border zone of a central infarction much faster than other functions. Still inside the cell you have a lot of lactate and hydrogen ions and a depletion of ATP and so on and it will take time to have them restored. I believe you get very fast changes in the ST-segment by beta-blockade and that is due to an immediate block of the membrane-labilizing effect of norepinephrine. But it will take quite a long time for the cell to reestablish functions like contractility. There will be a time lag from changing ST-segment to getting back all cell functions and I think this is the main problem. When Dr Braunwald is talking about the effects of hyaluronidase I think the important positive effect of the drug is to enhance diffusion of metabolites from the border zones to well perfused areas and not to enhance diffusion of substrates and oxygen to this zone.

EFFECT OF CARDIOSELECTIVE BETA BLOCKADE ON HEART FUNCTION AND CHEST PAIN IN ACUTE MYOCARDIAL INFARCTION

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SUMMARY

Systolic time intervals and the a/H ratio were recorded in 20 patients with uncomplicated acute myocardial infarction over a period of five days. The initial high heart rate and systolic blood pressure and the short PEP and ICT indicating a sympathetic overactivity were spontaneously normalized during the first week of infarction LVET was reduced indicating a fall in stroke volume and the a/H ratio was unchanged at the high levels suggestive of elevated preload or LVEDP

In 10 patients with acute myocardial infarction and recurrent chest pain recordings on noninvasive parameters were made before and 30 min after intravenous injection of practolol In addition 7 patients with chest pain classified as acute myocardial infarction, were given practolol. The average dose of practolol was 17 9 mg ranging from 5 to 30 mg. An almost immediate and pronounced relief of pain was observed in all patients and no signs of impaired left ventricular function appeared. The product of systolic blood pressure and heart rate was decreased by practolol and the PEP and the ICT were prolonged to normal values while no changes were seen in LVET and a/H ratio On 126 occasions practolol was given in dosages ranging from 5 to 30 mg (mean 8 mg) to 75 patients with acute myocardial infarction and recurrent chest pain. A satisfactory pain relief was seen on 108 occasions. It is suggested that an inappropriate sympathetic overactivity is an important factor in provoking recurrent chest pain in acute myocardial infarction Administration of the beta-adrenergic blocking agent practolol resulted in pain relief due to reduction of heart work and in severity of myocardial ischemia. The beta-blocking agent was well tolerated in the present study Continuous beta-blockade during the whole hospital stay to patients with acute myocardial infarction seems to be a very attractive therapy in order to preserve the ischemic myocardium and limit the *size of infarction*

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INTRODUCTION

The efficacy of a modern coronary care unit today is so complete that death from arrhythmia alone is regarded as an error The correlation coefficient of mortality rate to the height of cardiospecific serum enzymes is regarded as a test of efficacy of coronary care (Chapman, 1971) Therefore further reduction of mortality in acute myocardial infarction can only be obtained if measures are taken to reduce the area of myocardial ischemia and the size of myocardial infarction Different pharmacological interventions in animal models have suggested the possibility of influencing myocardial infarction size (Maroko *et al* 1971 1973 Reimer *et al* 1973 Braunwald & Maroko 1974).

Of various factors found to reduce the size of an experimental myocardial infarction beta-blockade seems to be the most important one. The local release of noradrenaline in the ischemic area of the heart may be the trigger mechanism for the infarct process (Hjalmarson & Waldenström 1975). The present study was undertaken to study whether beta-blockade could reduce ischemic chest pain in patients admitted to the coronary care unit. The positive effect of beta-blocking agents in angina pectoris is well documented (Fitzgerald 1977). On theoretical grounds a cardioselective beta-blocking agent might be better tolerated in acute myocardial infarction since it will not increase the peripheral vascular resistance in patients with an elevated sympathetic activity or induce bronchial obstruction in patients with lung disease The cardioselective beta-blocking agent practolol (Eraldin, ICI) has been given intravenously to patients during the first day of myocardial infarction The effect of practolol was studied on ischemic chest pain and on heart function as measured by noninvasive parameters

Table I Description of patient with uncomplicated acute myocardial infarction (AMI) or chest pain.

	Untreated AMI		Practolol-treated AMI
	Subgroup A	Subgroup B	
Number of patients	10	10	10
Age (years)	54 6 ± 1 9	53 9 ± 1.8	56 0 ± 2.5
GOT_{max} (IU/l)	177 ± 28	96 ± 16	108 ± 17
GTP_{max} (IU/l)	26 ± 4.5	25 ± 4 1	19 ± [illegible]
SR_{max} (mm/hr)	37 ± 11	65 ± 9.2	59 ± 1
$Temp_{max}$ (°C)	38 1 ± 0 17	38 4 ± 0 14	38 4 ± 0
Day with elevated morning temperature	6 ± 1.6	6 8 ± 1.3	7 4 ± 1.8
Heart size (ml/m² body)	452 ± 26	466 ± 17	515 ± 26

Laboratory data, body temperature and chest x-ray findings in two groups of patient with acute myocardial infarction, one untreated (subgroups A + B) and one treated with practolol. All values are mean ± SE. (Reproduced by permission from the Editor of Br Heart J 36 1111 1974)

including apex cardiogram carotid pulse tracing phonocardiogram and electrocardiogram.

MATERIALS AND METHODS

Two different studies were included in the present study

Study No 1

Patients with chest pain and suspected acute myocardial infarction were studied in the coronary care unit as soon as possible after arrival and not later than 48 hours after the onset of chest pain Apex cardiogram carotid pulse tracing phonocardiogram and electrocardiogram were recorded simultaneously in 55 patients To be included in the study the patients had to fulfil the following criteria 1) no previous myocardial infarction) ell defined onset of chest pain 3) no clinical vidence of heart failure 4) systolic blood pressure abo e 100 mm Hg 5) heart rate not lower than 45 beat min 6) no rrhythmia requiring therapy (atrial fib lation more than five ventricular beat m n no atrioventricular block 8) no tre tment th digitalis diuretics catecholamines atropin ntiarrhythmic drugs other than lignocain 4 patient were given lignocaine) and 9) no QRS d ration above 100 msec

Time m p t t th un omplicated acute m oca dial m

The aim of th part of the tudy was to follow patients with a t myocardial infarction with noninvasive technique for f e days During the first two days 25 patient were excluded from the study for various re son 5 patients did not fulfil the WHO criteria of acut myocardial infarction and 20 because they failed the above criteria in one way or another Twenty patient with acute myocardial infarction were studied five times at 4 hour intervals. These patients were divided into two groups. subgroup A studied within 4 hours after onset of chest pain and subgroup B studied 4 to 48 hours after onset of pain. All patients except one had transmural myocardial infarction of considerable size (Table I)

Effect of practolol on noninvasive pa ameters and chest pain in patients

In 10 patients with acute myocardial infarction and recurrent chest pain (Table I) recordings of noninvasive parameters were made before and 30 min after intravenous injection of practolol In addition 7 patients with chest pain classified as acute myocardial infarction were given practolol The average intravenous dose of practolol was 17 9 mg and doses of 5 to 30 mg were given Practolol 5 or 10 mg was injected rapidly at a time and this was repeated after one min if necessary The time lag between the injections of analgesics and practolol was at least 30 min for an intravenous dose and 60 min for an intramuscular dose Pain was graded on a scale from 0 to 5 (Table II)

Technique of egi tration and evaluation of curves

The systolic time intervals and the a/H ratio were measured f om simultaneously recorded electrocardiogram phonocardiogram, carotid pulse tracing and apex cardiogram A direct writing four-channel ink jet Mingograph 34 (Elema-Schönander) with a paper peed of 100 ± 0.5 mm/sec was used Electrocardiogram lead II was always used A piezoelectric microphone (Elema-Schönander) was placed at the left sternal border in such a position as to obtain the best recording of the second heart sound The signal was passed through a filter giving a response f om 1 .5 to 800 Hertz with relative damping of the lower f equencies to identi-

Table II Effect of practolol on recurrent chest pain in acute myocardial infarction. (Reproduced in part by permission from the Editor of Br Heart J 36: 1114 1974.)

Grading of pain:

Grade 0	No pain.
Grade 1	Retrosternal oppression.
Grade 2	Easy constant retrosternal pain without radiation not requiring analgesics.
Grade 3	Moderate constant retrosternal pain without radiation requiring analgesics. The patient does not show any signs of pain.
Grade 4	Intense constant retrosternal pain with radiation. The patient shows signs of pain.
Grade 5	Very intense retrosternal pain with radiation. The patient is pale, cool, sweating, frightened, restless and often screaming.

17 patients with chest pain.
Practolol dose average 17.9 mg (5-30 mg)
Degree of pain before 3.92±0.23 (p <0 001)
after 0.88±0.33

fy the initial part of the aortic closure sound more accurately The carotid pulse tracing and apex cardiogram were recorded by a special funnel-shaped pick-up connected with an air-containing rigid latex tubing of an Elema-Schönander EMT 510 C crystal transducer The system had a frequency response from 0 08 to 60 Hertz (3 dB) and a time constant of 1 9 to 3.8 sec depending on the amplification

The recordings were made with the patient in the left lateral position with the left arm abducted. The pick-ups were firmly pressed against the right common carotid artery and the point of maximal precordial movement. All recordings were made during apnoea after a normal expiration The Valsalva manoeuvre was avoided. Only records showing a well-defined upstroke and incisure in the carotid pulse tracing and a well-defined O point, a-wave and systolic upstroke in the apex cardiogram were used. Care was taken to obtain curves of comparable amplitude from one time to the next in order to avoid distortions A transducer with a minimum time constant close to 2 sec was used

The patients were examined carefully before all recordings and after injection of practolol Auscultation of the lungs was performed to check for rales and an apical phonocardiogram was recorded for possible third and fourth heart sounds Patients were questioned concerning dyspnoea and chest pain. If necessary a bedside chest x-ray was taken. To estimate the heart size all patients had a chest x-ray taken after mobilization in the standing position.

From simultaneous electrocardiogram phonocardiogram, carotid pulse tracing, and apex cardiogram the following measurements were obtained. The left ventricular ejection time (LVET) is the interval from the carotid upstroke to the incisure notch The preejection period (PEP) is QS_2-LVET where S_2 begins with the initial high frequency vibration of the aortic closure sound *The pulse transmission time* (PTT) is the interval from S_2 to incisure notch, which is suggested to be the same at the beginning and end of the ejection period. The isometric contraction time (ICT) is PEP EMI (Inoue *et al* 1970; Willems *et al* 1971) The a/H ratio is the ratio between the height of the a-wave and the total height of the apex cardiogram (H) measured in per cent. In most cases it was easy to determine the systolic upstroke In a few cases the a-wave was very close to the systolic upstroke of the apex cardiogram and the point after the a wave showing the lowest angle of the tangent was used as the beginning of isometric contraction.

LVET was corrected for heart rate in two ways. 1) the LVET index was calculated i.e. based on the fact of a linear relation between LVET and heart rate according to Weissler *et al* (1969) and 2) according to Meiners diagram (Meiners 1958) which

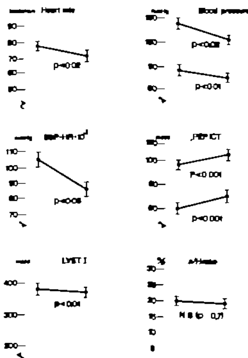


Fig. 1 Heart rate blood pressure product of systolic blood pressure and heart rate preejection period (PEP) isometric contraction time (ICT), left ventricular ejection time index (LVETI), and a/H ratio on day 2 and 5 after onset of chest pain in 20 patients with acute myocardial infarction. Values are mean ± SE. (Reproduced by permission from the Editor of Br Heart J 36 1115 1974.)

is based on an exponential relation between LVET and heart rate (relative LVET) A better regression line coefficient was found for the exponental compared to the linear function (Meiners 1958 Willems & Kesteloot 1967) PEP was given both as an absolute value and corrected for heart rate (Harris *et al* 1967 Talley *et al* 1971 Weissler *et al* 1965) ICT was given without correction All values are means of those derived from five consecutive heart beats. The heart rate was calculated from the electrocardiogram

Study No 2

Seventy-five patients were given practolol for recurrent chest pain on 126 occasions in doses ranging from 5 to 30 mg intravenously until effect on chest pain was obtained None of the patients had signs of left ventricular failure such as bilateral basal rales blood pressure below 100 mm Hg systolic signs of low output with poor peripheral circulation regardless of blood pressure heart rate below 45 beats/min and AV-block I II or III If satisfactory effect was seen on chest pain the patient was classified as responder and if additional analgesics had to be given the patient was classified as non-responder The patient were divided in groups according to time relapse from onset of chest pain to beta-blocking therapy for recurrent chest pain and according to the dose of beta-blockers

RESULTS

Noninvasive measurements during 5 days in patients with acute myocardial infarction

Noninvasive measurements recorded on 5 consecutive days in 20 patient with acute myocardial infarction (Fig 1) showed a significant fall in heart rate blood pressure product of systolic blood pressure and heart rate and rate-corrected left ventricular ejection time whereas preejection period and isometric contraction time were significantly prolonged. The a/H ratio was unchanged at a high level The PEP/LVET increased significantly (Fig.)

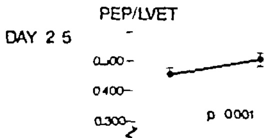


Fig 2. PEP/LVET ratio in 20 patients (subgroups A B) with uncomplicated myocardial infarction on day and 5 after onset of chest pain (Reproduced in part by permission from the Editor of Br H art J 36 1116 1974)

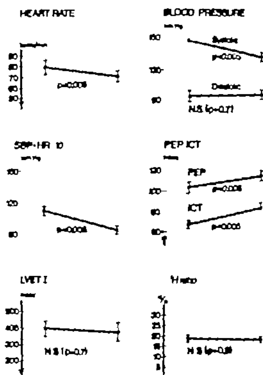


Fig 3 Heart rate blood pressure product of systolic blood pressure and heart rate preejection period (PEP), isometric contraction time (ICT), left ventricular ejection time inde (LVETI), and a/H ratio in 10 patient with acute myocardial infarction before and 30 min after intravenous injection of practolol in an average dose of 19.5 mg. Values are mean ± SE. (Reproduced by permission from the Editor of Br Heart J 36, 1116 1974)

Effect of practolol on noninvasive measurement in patients with acute myocardial infarction

Practolol given intravenously to 10 patients with acute myocardial infarction and chest pain on the first day of infarction in doses averaging 19.5 mg (5-30 mg) gave a significant fall in heart rate systolic blood pressure product of heart rate and systolic blood pressure PEP and ICT whereas LVET and a/H ratio were unchanged (Fig. 3) PEP/LVET ratio was slightly prolonged (Fig 4) but not as much as in the group of untreated acute myocardial infarctions (Fig).

Effect of practolol on chest pain

Grading the pain from 0 to 5 (Table II) practolol gave significant reduction of chest pain in the 10 patients in whom noninvasive measurements were

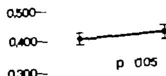


Fig 4 PEP/LVET ratio obtained in 10 patients with acute myocardial infarction before and 30 min after dose of practolol given intravenously for chest pain on day 1 of infarction. Values are mean ± SE. (Reproduced in part by permission from the Editor of Br Heart J 36 1116 1974)

done and another 7 where only registration of pain was done. The doses of practolol ranged from 5 to 30 mg (17 9 mg), and the total dose was given within 8 min. The pain relief was obvious within 2 min in all patients, but the maximal effect was seen after 10 min and lasted more than 30 min. In some of the cases pain relief had not been obtained by analgesics given more than 30 min before practolol, but a prompt relief of pain was nevertheless seen after practolol

Effect of practolol on ecurrent chest pain

On 126 occasions 75 patients with acute myocardial infarction and recurrent chest pain were given practolol in doses ranging from 5 to 30 mg. A satisfactory pain relieving effect was seen on 108 occasions whereas 18 cases were classified as non-responders Most patients were given a dose of 5 mg (Fig. 5) and an even distribution of responders and non-responders was found during the

Table III. Practolol on recurrent chest pain in acute myocardial infarction.

	Responder (= 108)	Non-responder (n = 18)
Heart rate	93 ±2	92 ± 5
Systolic blood pressure	158 ±3	158 ± 9
Diastolic blood pressure	98 ±2	98 ± 5
Heart rate and systolic blood pressure	145 ±4	147 ±13
Dose of practolol	7 9±0 5	8.0± 1.3

Heart rate blood pressure product of systolic blood pressure and heart rate and dose of practolol in 75 patients on 126 occasions. Mean ± SE.

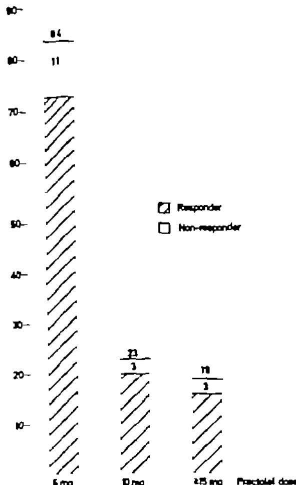


Fig. 5 Practolol on recurrent chest pain in acute myocardial infarction. Distribution of 126 treatments in 75 patients according to dose of practolol.

first 72 hours of myocardial infarction (Fig. 6) Since the patients were selected due to a high heart rate and/or high systolic blood pressure the average values for these parameters were quite high and no difference between the responders and non-responders was seen (Table III).

DISCUSSION

During the first days of acute myocardial infarction heart rate and systolic blood pressure are higher and PEP and ICT are shorter compared to days 5 and 6 after onset of chest pain and to normal subjects in agreement with others (Jain & Lindahl 1971 Fabian *et al* 1972) A prolonged PEP has been reported in acute myocardial infarction (Diamant & Killip 1970) that might be due to congestive heart failure which is known to prolong PEP (Weissler

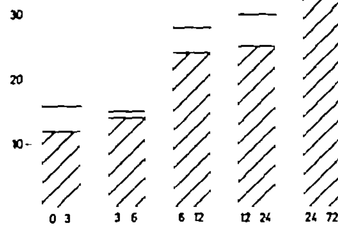


Fig. 6 Practolol on recurrent chest pain in acute myocardial infarction. Distribution of 126 treatments in 75 patients according to time from onset of chest pain.

et al 1968) Only small changes were seen in diastolic blood pressure in both groups and a/H ratio was unchanged on a high level suggesting unaltered high filling pressure (Voigt & Friesinger 1970; Kahn *et al* 1977) The changes in ICT and PEP were interpreted as a decrease in the rate of left ventricular pressure development (Talley *et al* 1971) The fall in LVET during the first days of myocardial infarction indicates a decrease in stroke volume and the increase in the PEP/LVET ratio reflects depressed left ventricular function (Garrard *et al* 1970; Ahmed *et al* 19) The changes in heart rate blood pressure and systolic time interval during the first day of acute myocardial infarction are suggested to be due to initial inappropriate sympathetic over-activity which is spontaneously reduced during the first week of infarction This is supported by the observation that there are high levels of circulating catecholamines in the early stage of myocardial infarction (Gazes *et al* 1959 Valori *et al* 1967 Wallace 1968) During the first week of infarction there is a stepwise decrease in urinary catecholamine excretion (Wallace 1968) The increase in PEP during the first week of myocardial infarction in this study could be due to reduction of LVEDP although not very likely since the a/H ratio was unchanged at an elevated level The significantly higher a/H ratio of the apex cardiogram in the early stage of infarction has earlier been reported (Jain & Lindahl 1971) and good correlation has been found between LVEDP and a/H ratio in patients who have had myocardial infarction or heart failure for other reasons (Rios & M umi 1965 Epstein *et al* 1968 Voigt & Friesinger 1970; Kahn *et al* 1977)

An intravenous injection of the selective beta-adrenergic blocking agent practolol on the first day of myocardial infarction reduced heart rate and systolic blood pressure prolonged PEP and ICT and increased PEP/LVET while a/H ratio was unchanged at a high level The injection of practolol induced similar changes as occurred spontaneously during the first week of myocardial infarction in untreated patients Thus practolol abolished the increased sympathetic activity during the early stage of infarction The reduction of heart work by practolol was also demonstrated by a pronounced decrease in the degree of chest pain This supports the hypothesis that beta-adrenergic blockade in acute myocardial infarction might diminish the severity and extent of myocardial ischemia and could be used in order to limit the size of myocardial infarction as suggested in animal experiments (Maroko *et al* 1971 Libby *et al* 1973 Waldenström & Hjalmarson 1975) LVET was unchanged in most patients given practolol indicating that the stroke volume was not reduced (Braunwald *et al* 1958 Weissler *et al* 1961 Wallace *et al* 1963 Fabian *et al* 1977). This is in agreement with studies by Jewitt *et al* (1970) who found unaltered or even increased stroke volume after practolol to patients with acute myocardial infarction Practolol did not aggravate congestive heart failure in the patients with uncomplicated infarction as was demonstrated by an unchanged high a/H ratio at the early stage of infarction No clinical sign of manifest left ventricular failure appeared in

the present study after a single injection of practolol is agreement with others (Jewitt et al 1970 Jewitt & Croxson 1971) The beta-blocking agent propranolol which is not cardioselective and may increase peripheral resistance under some conditions has been reported to reduce LVET and thus stroke volume in man (Harris et al 1967) Recurrent chest pain in acute myocardial infarction is quite common during the first 24 hours after onset of chest pain and is also accompanied by elevated blood pressure and heart rate It was found that practolol had a very good pain-relieving effect even in small dosages on recurrent chest pain and was in some cases superior to conventional analgesics The good pain-relieving effect by practolol on recurrent chest pain in this study was confirmed in a controlled study of initial chest pain in acute myocardial infarction when beta-blockers including practolol were compared to placebo in a double-blind way (Waagstein & Hjalmarson, 1975).

The cardioselective beta-blocking agent practolol has been found to reduce heart work and ischemic chest pain in this study Furthermore it has been found to reduce the ST segment elevation in acute myocardial infarction indicating a reduction in the degree of myocardial ischemia (Pelides et al 197? Waagstein & Hjalmarson, 1975) It seems likely that beta-blockade in patients with acute myocardial infarction might reduce the severity and extent of myocardial ischemia and limit the size of infarction as shown in animal experiments (Maroko et al 1971 Libby et al 1973 Waldenström & Hjalmarson, 1975). In studies using the microsphere technique beta-blockade was found to reduce the ST segment elevation although it reduced also blood flow indicating that beta-adrenergic blocking agents do not alter ischemia through changes in blood flow distribution (Becker et al 1975). The *ST segment elevation is most likely* due to local release of noradrenaline which is suggested to be a most important factor for development of myocardial infarction and initiation of ventricular fibrillation (Ceremuzyński et al 1968, Khan et al 1972, Hjalmarson & Waldenström 1975). This idea is supported by a clinical observation that practolol given to patients who have had a myocardial infarction reduced the over all mortality sudden death and the number of reinfarctions (Ahlmark et al 1974 Wilhelmsson et al., 1974 A Multicentre International Study 1975). Thus several findings suggest a very positive protective effect of beta-blockade in acute myocardial infarction in man and it seems at present to be the most promising therapy for preservation of the ischemic myocardium and for limitation of the size of myocardial infarction The present study encourages to further studies with continuous beta blockade during the whole hospital stay and such studies are in progress at our hospital

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DOUBLE BLIND STUDY OF THE EFFECT OF CARDIOSELECTIVE BETA BLOCKADE ON CHEST PAIN IN ACUTE MYOCARDIAL INFARCTION*

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SUMMARY

A double-blind study including three different car dioselective beta-blockers practolol H 87/07 and metoprolol was performed in 54 patients with acute myocardial infarction and chest pain shortly after onset of symptoms. Transmural infarctions were found in 42 patients while 12 patients had non-transmural infarctions Chest pain and the product of heart rate and systolic blood pressure were significantly reduced in the beta-blocker groups whereas no changes were seen after saline All patients with nontransmural infarctions and 14 out of 29 with transmural infarctions got pain relief lasting for at least 30 min. None of the patients developed signs of left ventricular backward failure shock or bradycardia. A decrease in ST segment elevation was observed in all the transmural infarctions after beta-blockade No changes in ST segment elevation were found after analgesics when given after saline but in some cases an increase was seen in this parameter when analgesics were given due to insufficient pain relief after beta-blockers or due to return of chest pain It is suggested that pain relief by beta-blockers indicates decrease of myocardial ischemia.

INTRODUCTION

In experimental myocardial infarction factors de creasing heart work were found to reduce size of infarction (Maroko *et l* 1971) Beta-blockade was considered a factor of great importance (Libby *et al* 1973 Waldenström & Hjalmarson, 1975) In a previous study practolol given to patients with acute myocardial infarction was found to reduce cardiac work and recurrent chest pain (Waagstein *et al* 1974). The same observation was made by Mueller *et al* (1974) who in addition found an improved aerobic utilization in the myocardium The observation that practolol could reduce re current chest pain during the early phase of acute myocardial infarction was made from an open study (Waagstein *et al* 1974) It was therefore considered important to repeat this study double-blind in patients with acute myocardial infarction and initial chest pain Because of various reports on serious side-effects of practolol two other cardioselective beta-blockers were also used. H 87/07 has a slightly higher intrinsic stimulatory activity compared to practolol and metoprolol has no intrinsic stimulatory activity (Åblad *et al* 1973) In some patients the effect of the beta-blocking agents was studied also on ST seg ment elevation

MATERIALS AND METHODS

Patients admitted to the hospital with clearcut elec trocardiographic evidence of acute myocardial infarction and still suffering from chest pain of mode rate to severe degree were taken into the study if the following criteria were fulfilled. 1) no treatment with beta-blockers within the last 4 hours 2) no clinical signs of left ventricular backward failure such as bilateral lung rales and/ or severe dyspnea, 3) systolic blood pressure above 100 mm Hg, 4) no sign of poor peripheral circulation with coldness and palor regardless of blood pressure 5) heart rate more than 45 beats/ min, and 6) no AV-block I II or III Previou treat ment with digitalis and/or diuretics for heart de-compensation was no contraindication if the pa tient was compensated

The material consisted of 54 patient 1 with nontransmural and 4 with transmural infarcti n All patients except two suffered from their first acute myocardial infarction and were divided ran domly into four groups (Table I) 1) 15 pa

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Table I Age GOT_{max} GPT_{max} SR_{max} $temp_{max}$ days with morning temperature and heart size after mobilization in saline and the three beta-blocker groups

	Saline	n = 15	Practolol	n = 18	H 87/07	n = 1	Metoprolol	n = 9	Total	n = 34
Age (years)	6_1	± 1 8	60 7	± 1 9	61 9	± 1.8	65 0	± 4.9	6_0	± 1.5
GOT_{max} (IU/l)	105	± 15	107	± 30	103	± 79	1_5	± 22	108	± 12
GPT_{max} (IU/l)	17	± 4 4	70	± 5_	26	± 5 1	_3	± 4 0	_3	± [illegible]
SR_{max} (mm/hr)	39	± 10	61	± 16	52	± 10	60	± 1	48	± 5
$Temp_{max}$ (°C)	38 0	± 0 1	38	± 0 1	38 3	± 0_	38.3	± 0_	38.2	± 0 1
Days (>37)	8	± 0 9	3 6	± 1 0	4 4	± 1 0	3 7	± 1 1	3.6	± 0 5
Heart size (ml/m² body)	484	± 31	445	17	44_	± 25	494	± 57	461	± 15

Mean ± SE

given 15 ml saline) 18 patient were given practolol 15 ml (30 mg ICI) 3) 1 patient were given H 87/07 (30 mg AB H ssle Sweden Fi 1) and 4) 9 patients were given metoprolol (H 93/26) 15 ml (15 mg AB Häs le Swede F g 1) The mount of beta-blockers given w nposed to be equ potent with regard to the bet block ng effe t H 87/07 (Fig 1) is a selecti be ke w th m re intrinsic stimulatory act r d to pr ct lol Metoprolol (Fig 1 h -blocker which lacks intrin t Åblad t al 1973)

Patient w tion ECG was recorded t writ ng ink jet four-ch. M ph 34 Flema Schönand f 10 mm/sec in terrupt d r od with a paper peed f rd for ST segment me m p tient with predom na t rfarction four precor d l le \ \ were used In patient with h l nfarct on leads II III and aVF wer d l le d howing the maximal ST segment lev t on was chosen and the amplification w s set t g e at least 5 mm elevation of the ST segment if possible The reason for doi g thi w that only changes in ST segment were considered The ST segment deviations were mea red us ng the TP segment as the isoelectric line or the PQ segment when the TP segment wa difficult to locate because of tachycardia. The ST segment was measured to the nearest 0.5 mm 0 06 sec after the end of the S-wave (Reid *et al* 1971). The values of ST segment elevations were calculated as the mean of at least 10 beats. Heart rate was measured from ECG and blood pressure was recorded indirectly with a cuff Chest pain was graded according to a scale ranging from 0 to 5 (Table II)

After a control period which for ethical reasons was very short (about 3 min) the patients were given injections of 5 ml (saline or beta-blockers in solution) rapidly intravenously with an interval of about 1 min In total 15 ml was given. Blood pressure and heart rate were checked every min. The injection was stopped if heart rate was below 45 beats/min or systolic blood pressure was below 100 mm Hg. Blood pressure and heart rate were then recorded and the degree of chest pain was estimated 5 10 20 30 45 and 60 min after end of injection Degree of chest pain heart rate blood pressure and ST segment elevation 5 min after injection was used for estimation of the effect of the injection. Pain degree 2 or lower was considered satisfactory whereas pain degree 3 or more required additional analgesics and was regarded as unsatisfactory pain relief If chest pain was graded

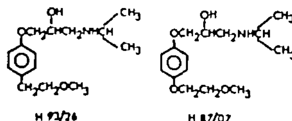


Fig 1 Structural formula for metoprolol (H 93/26) and H 87/07

Table II Grading of pain in patients with acute myocardial infarction.

Grade 0	No pain.
Grade 1	Retrosternal oppression.
Grade	Easy constant retrosternal pain without radiation not requiring analgesics.
Grade 3	Moderat constant retrosternal pain without radiation requiring analgesics. The patient does not show any signs of pain.
Grade 4	I tensiv constant retrosternal pain with radiation The patient show signs of pain.
Grad 5	Very intense retrosternal pain with radiation The patient is pale cool sweating, frightened, restles and often screaming

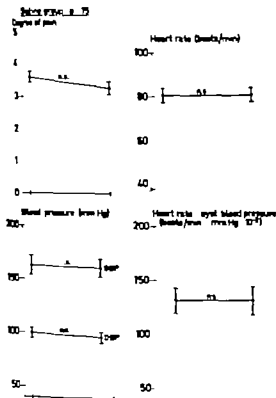


Fig. 2. Chest pain, heart rate, blood pressure and product of heart rate and systolic blood pressure before and 5 min after injection of saline.

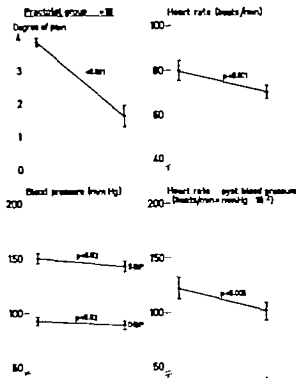


Fig. 3 Chest pain, heart rate blood pressure and product of heart rate and ystolic blood pressure before and 5 min after injection of practolol (30 mg).

to 3 or more 5 min after end of injection analgesics were given intravenously Control values for heart rate, blood pressure and ST segment were taken. Estimation of ST segment elevation was repeated after 10 min. If the patient after satisfactory pain relief had a new attack of pain ST segment was measured again. Patients with more than 5 multifocal ventricular extra beats (VEB) per min and ven tricular tachycardia (VT) more than 3 beats were given lidocaine 50-100 mg intravenously as a bolus and -4 mg/min as infusion.

RESULTS

Effect on heart rat and blood press e

No significant changes were seen in heart rate and blood pressure in the saline group (Fig. 2). A significant decrease in heart rate was observed in the beta-blocker groups (Fig. 3-5). Average fall was greatest after metoprolol followed by practolol and H 87/07 In none of the cases a fall in heart rate below 45 beats/min was seen after the total dose of beta-blockers. In no case injection was stopped before full dose of beta-blockers had been given. There was a significant fall in systolic blood pressure after metoprolol and practolol and in diastolic blood pressure only after metoprolol (Fig. 5) External heart work, expressed as the product of heart rate and systolic blood pressure was thus decreased by each of the three beta-blockers (Fig. 3-5). At 60 min after end of injection of beta-blockers there were no changes in heart rate or blood pressure compared to 5 min after injection.

Effect on chest pain

The degree of chest pain was significantly reduced in all three groups of patients given beta-blockers (Fig. 3-5). No significant pain relief was observed in the saline group Effect on chest pain was seen within 2 min after end of injection and maximum effect was reached about 5 min after completed injection. The degree of pain 5 min after the injection is given in the figures. Satisfactory pain relief was achieved in 28 out of 39 patients when considering a 10 min period after end of injection. Another four patients (Table III) required analgesics 10-30 min after end of injection due to return of chest pain, which nearly always was of lower intensity com-

segment was seen after all three beta-blockers but not after saline (Table IV). Analgesics given after saline showed no decrease in ST segment (Table IV). When analgesics had to be given either due to failure of the initial beta-blocker injection to give sufficient pain relief or due to return of chest pain, surprisingly an increase in 8 of 13 cases was seen despite relief of pain (Table V). Decrease in ST segment elevation was seen in some cases without pain relief. On the other hand, pain relief was never seen without decrease in ST segment except for a slight relief in two patients in the saline group.

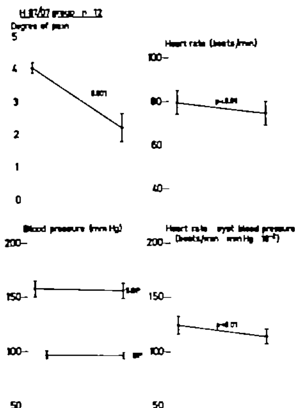


Fig. 4. Chest pain, heart rate, blood pressure and product of heart rate and systolic blood pressure before and 5 min after injection of H 87/07 (30 mg).

pared to the initial chest pain. Five patients required analgesics 30-60 min after end of injection for the same reason (Table III). Thus, a satisfactory pain relief was obtained during the first 60 min after completed injection of beta-blockers in 19 out of 39 patients. All patients with nontransmural infarctions had satisfactory pain relief (Table III). Mean heart rate before beta-blockers was the same in the group with satisfactory pain relief as in the group which did not respond to beta-blockade. However, the fall in heart rate after beta-blockade was 12.1 beats/min in the responder group but only 6.4 beats/min in the group which did not respond satisfactorily to beta-blockers.

Effect on ST segment

In 31 out of 54 patients it was possible to measure changes in ST segment after the initial injection of beta-blockers or saline (Table IV). In 23 patients this was not possible due to nontransmural infarctions or bad quality of registration. A decrease in ST

Side-effects

Very few side-effects were seen which could be referred to beta-blockers. None of the patients developed left heart backward failure. One patient in the metoprolol group had a drop in systolic blood pressure from 125 to 80 mm Hg and a fall in heart rate from 66 to 59 beats/min. The patient was given atropine 0.5 mg intravenously and an increase in blood pressure to 115 mm Hg and heart rate to 80 beats/min was seen within 2 min. Nausea was

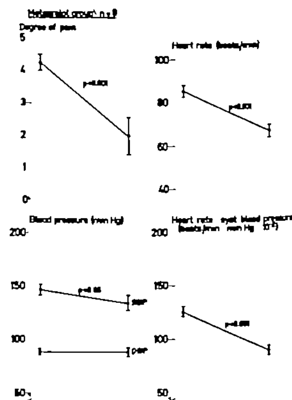


Fig. 5. Chest pain, heart rate, blood pressure and product of heart rate and systolic blood pressure before and 5 min after injection of metoprolol (15 mg).

Table III Distribution of transmural and nontransmural infarction in relation to response on chest pain and time until first injection of analgesics had to be given.

Injection	Type of infarction	n	Satisfactory pain relief	Analgesics		
				5 min after injection	10-30 min after injection	30-60 min after injection
Saline	transmural	13	0	13	0	0
total 15	nontransmural	2	0	2	0	0
Practolol	transmural	12	8	4	2	1
total n = 18	nontransmural	6	6	0	0	0
H 87/07	transmural	8	5	3	2	1
total = 12	nontransmural	4	4	0	0	0
Metoprolol	transmural	9	5	4	0	3
total 9	nontransmural	0	0	0	0	0

observed in one patient given H 87/07 However nausea was a common finding after analgesics

DISCUSSION

The present study shows that considerable relief of chest pain and decrease of ST segment elevation can be obtained when a selective beta-blocker is given intravenously shortly after onset of chest pain in patients with acute myocardial infarction. If the patients were found suitable for beta-blockade on the basis of clinical findings and ECG no signs of left ventricular backward failure shock, bradycardia, or AV-block developed after beta-blockade The good tolerance to beta-blockers could not be explained by small infarctions since 42 of the 54 patients had transmural ones Furthermore average GOT_{max} value suggests acute myocardial infarctions of considerable size (Witteveen *et al* 1975). Heart rate was reduced by the three beta-blockers. The greatest fall was seen after metoprolol, which lacks intrinsic stimulatory activity followed by practolol, which had some intrinsic activity and H 87/07 with the highest intrinsic activity (Åblad *et al* 1973). The different properties of the beta-blockers were reflected in the changes in systolic blood pressure showing a fall in the metoprolol and practolol groups but no significant change in the H 87/07 group

Table IV Change in ST segment elevation in patients with chest pain and acute myocardial infarction after intravenous injection of saline analgesics and different beta-blockers.

Injection	%	n
Saline	1 9 ±1 7	10
Analgesics after saline	0 7 2 3	10
Practolol	29 0 ±9 2	6
H 87/07	31 6 ±6 4	8
Metoprolol	-40 0 ±6 2	7
Analgesics after beta-blockade	+17 53±6 3	13

Mean ± SE

Chest pain was used as an index of myocardial ischemia since it is caused by ischemic changes in the heart and it is the most obvious clinical symptom in acute myocardial infarction. A parallel between ST segment elevation and chest pain was found in Prinzmetal's angina (Prinzmetal *et al* 1959; Guazzi *et al* 1975) Since none of the three beta-blockers possesses a membrane stabilizing property which means potential local anesthetic activity reduction in chest pain was interpreted as decreased myocardial ischemia. The effect on chest pain was about the same in the three groups of beta-blockers. Initial pain was somewhat higher in the metoprolol group compared to the two others This could possibly be explained by the fact that all the 9 patients in the metoprolol group had transmural infarctions but only 8 out of 12 in the H 87/07 group This is also reflected by higher average values for GOT in the metoprolol group The best effect by beta-blockade on chest pain should be expected when acute myocardial infarction is nontransmural.

The sum of ST segments reflects the degree of myocardial ischemia and predicts the area of necrosis (Maroko *et al* 1972) In animal experimental models practolol has been shown to decrease the mean ST segment and the sum of ST segments and even reduce the amount of necrotic myocardial tissue Surface mapping of ST segment in man has shown decrease in a number of sites with ST elevation and decrease of mean ST elevation (Pelides *et al* 1972). In the present study only the lead showing maximal ST segment elevation was used. However when comparing per cent changes in the lead showing maximum ST segment elevation with per cent changes in four precordial leads a good correlation was seen (Wangstein unpublished ob-

Table V Per cent changes in ST segment elevation after analgesics in relation to changes in heart rat blood pressure occurrence of arrhythmias, and amount of analgesics in patients with acute myocardial infarction initially given beta-blockers.

Patient	% Change in ST segment	Heart rate		Blood pressure		Other conditions of importance for myocardial O_2-consumption or delivery
		before	after	before	after	
1	+53	84	84	120/ 95	120/ 95	High dose of analgesics.
2	−18	85	97	145/100	150/100	VEB in bigemini before analgesics which disappeared after treatment with lidocaine
3	− 2	80	80	140/100	140/100	–
4	+11	112	116	150/ 95	150/ 95	–
5	+57	75	75	180/115	230/125	–
6	+ 4	60	60	125/ 95	130/100	–
7	+43	72	76	120/ 85	130/ 95	High dose of analgesics.
8	+23	71	71	125/ 90	125/ 90	High dose of analgesics.
9	− 2	80	79	115/ 80	130/ 80	–
10	+16	80	81	145/100	145/100	–
11	+27	63	81	150/ 90	135/ 85	Appearance of VEB
12	+15	76	77	110/ 85	115/ 90	High dose of analgesics.
13	+ 1	74	74	120/ 85	115/ 85	–

servations). It is therefore believed that the directional changes observed in one lead reflect the changes which would have been seen from a surface mapping record Not all patients did reveal an ECG pattern with ST elevation of at least 1 mm on admittance to hospital This is not to be expected when considering the earliest electrocardiographic signs of myocardial infarction (Short, 1970) The natural history of ST elevation in acute myocardial infarction suggests that spontaneous disappearance of ST elevation is unlikely to occur during a 60 min period of observation (Mills *et al* 1975) No change was seen in ST segment after saline. This is expected since no change was seen in heart rate or blood pressure Pain relief in acute myocardial infarction is suggested to be of major importance However no change was seen in ST segment despite pain relief when analgesics were given after saline No change in heart rate or blood pressure was observed and since analgesics do not reduce contractility a decrease in myocardial oxygen consumption is not to be expected Significant changes in heart rate blood pressure or cardiac output have not been reported in acute myocardial infarction after morphine when given to a patient in supine position (Grendahl & Hansteen, 1969). The reason why the ST segment elevation was increased in 8 out of 13 cases when analgesics were given after beta-blockers is not clear However when studying Table V it seems reasonable that factors increasing myocardial oxygen consumption, such as higher heart rate or blood pressure or more frequent ventricular ectopic beats could be responsible at least in some cases High doses of morphine are known to depress ventilation and give some fall in arterial oxygen tension (Lal *et al* 1969) which could possibly deteriorate myocardial energy utilization. However the oxygen tension was not measured. It must be stressed that beta-blockers reduce myocardial ischemia resulting in pain relief while conventional analgesics, such as pethidine or morphine may reduce the pain without any favourable direct effects on the myocardium.

Depressed cardiac contractility after beta-blockers may not result in decreased cardiac output since a ventricular dilatation maintains stroke volume on geometric basis (Lekven *et al* 1973). In pacing induced angina there is a shift to the left of the log pressure volume curve suggesting an increase in diastolic tone probably due to failure of relaxation in the affected segment of myocardium in angina (Barry *et al* 1974). Therefore it seems reasonable to suggest that beta-blockers, when given early in acute myocardial infarction with ischemic chest pain will shift the log pressure volume curve to the right. This is consistent with that which has been observed after beta-blockade in patients with three vessel disease (Coltart *et al* 1975)

Catecholamines are suggested to play a central role in the development of myocardial infarction and the local release of noradrenaline from the sympathetic nerve endings may be most important (Hjalmarson & Waldenström, 1975). Absence of pain relief does not indicate that no benefit is to be expected from beta-blockers since the ST segment elevation was reduced in some patients with little effect on chest pain Moreover reduction of ventricular arrhythmias has been reported in acute myocardial infarction in man (Jewitt

et al 1969) and an increase in ventricular fibrillation threshold has been obtained after beta-blockers in experimental myocardial infarction of dog hearts *in situ* (Kliks *et al* 1975).

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DISCUSSION

Dr Braunwald

You said something about your current study Can you tell us what the experimental design is and what you are measuring and how you are following the patients

Dr Waagstein

The new study is a pilot study for later long-term study in order to see how the patient tolerates the prolonged beta-blockade because there could be some difference between giving a single dose and to have patient on continued beta-blockade We are measuring heart rate blood pressure and cardiac output by thermodilution and pulmonary artery pressure We have continuous ECG recording to see if there is any effect on arrhythmias. We are taking blood samples for metabolic analyses such as adrenaline noradrenaline, glucose lactate pyruvate and specific cardiac enzymes.

Dr Braunwald

Was there any correlation between the responders and the non-responders in regard to the electrocardiographic changes In other words was there a group that did not respond as far as pain is concerned

Dr Waagstein

Yes.

D Braunwald

Did the electrocardiogram show the same changes as in the responders

Dr Waagstein

As you see the number of cases was quite low and there were four groups We tried to make some analyses on that but I think the groups are too small. If you have 1 000 patients you might be able to say something about it We hope that there will be some correlation.

Dr Hjalmarson

It is obvious that the myocardial area we are working on is quite well perfused. It cannot be the most ischemic area where you have almost no perfusion at all. It has to be a border zone with moderate ischemia and ST-elevation but still a quite good coronary flow The beta-blocker will reach that area through the coronary arteries and an effect will be obtained in a couple of minutes on the ST segments and on the chest pain. In some patients there is a very dramatic change so I believe that what we talk about as the border zone is a quite well perfused zone, as Dr Thomas showed. This zone is moderately ischemic due to leakage of norepinephrine out from a more severely ischemic center or an unappropriate release of norepinephrine from sympathetic nerve endings.

Dr Thomas

ST-depression is a common concomitant in the clinical ischemic situation. In reference to that, if in acute experiments you reduce the lumen of a main stem coronary artery to a point that you get wide spread ST-depression the flow down that main stem coronary artery may be as little as 20 per cent of the original Radioactive microsphere studies show that collateral flow can increase the regional flow to as high as 90 per cent of the original so there again the flow is very much higher than one might at first hand anticipate (Timogiannakis G Amende T Martinez E. Thomas M (1974) Cardiovasc Res 8 No 4 469-477)

Dr Maroko

We have an interesting observation in relation to the penetration of drugs to the center of the infarct We studied, in collaboration with Drs Gold and Leinbach 9 patients with acute myocardial infarction to whom propranolol wa administered The patients who showed the most intense reduction in their ST segment elevations were those who did not have complete occlusions of their coronary arteries a seen by coronary angiography within a week of the acute event while those with complete occlusions and no collateral showed smaller reductions in the ST segments

Dr Braunwald

Dr Sobel at what point in the sequence of limitation of oxygen do you think that the cell loses its ability to retain CPK and what in your judgement actually happens when CPK leaks from a cell

Dr Sobel

In the late 1950 s Jennings examined myocardial enzyme loss in a model not too different from the one he is now using In general in his early studies and in many studies performed subsequently by others a well cell death appeared to accompany substantial loss of enzyme from the heart induced by an ischemic insult However the loss of enzyme may have no causal e role in mediating irreversible injury Rather the enzyme loss appears to be an index of the extent of damage The concept that a membrane alteration can lead to enzyme release not associated with irreversible damage is certainly tenable For example after vigorous exercise marked serum CPK elevations are frequently observed despite the absence of irreversible skeletal muscle injury Release of enzyme in these circumstances is not due to an ischemic insult but rather to subtle cellular alterations not tantamount to cell death. Similarly incubation of myocardium in calcium-free media leads to massive release of enzymes without corresponding cell death However when enzyme loss is a result of ischemia, and the ischemia is persistent, the release of enzyme appears to be tantamount to cell death.

By the same token the presence of transient ST-elevation by itself in a patient obviously does not imply cell death However when ST-elevation persists for several hours it frequently is associated with cell death (unless pericardial inflammatory processes or other metabolic alterations are responsible) The time-intensity product if you will of the ischemic insult, implies death of some cells. Although the relationship between either parameter and cell death may not be direct in a defined setting alterations in the value of the parameter are indicative of a qualitative change

With projected enzyme values which I did not discuss extensively we have analogous problems. The projections simply imply that under conventional circumstances with a typical evolving infarction one can make an educated guess regarding enzyme values anticipated a few hours later An intervention that alters observed compared to anticipated values may be judged to be directionally favourable or directionally unfavourable with respect to protection of myocardium, but proof requires correla tion of the altered enzyme values with other parameters measurable in experimental animals Thus an inference is involved when results in patients are extrapolated from those in experimental animals However although we do not have enough information yet to infer linear or direct relations and although we have not yet developed physiological models that describe the responsible processes pre cisely the implication that we cannot detect direc tional changes is not valid A great deal of evidence from several sources suggests that as long as conditions are carefully defined one can estimate direc tional changes meaningfully The question should be: is the intervention good or is it bad? It now seems that it is 'bad' to have a big infarct and 'good' to have a small one. If an intervention results in changes that reflect smaller rather than larger infarcts then presumably the intervention merits consideration and further study

Dr Bra ald

We heard from Dr Waagstein that he is in the group in Göteborg where they are starting a major trial of beta-blockers in acute myocardial infarction. I guess we will anxiously await the outcome of that. There has been a major trial going on as many of us know in the U K Dr Green is here and I wonder if he can give us any information.

Dr Green

A controlled multicentre double-blind trial of prac tolol versus placebo has recently been completed (Kenneth G Green Clinical Research Department, I C.I Ltd Pharmaceuticals Division Macclesfield Cheshire England Long-term prophylactic treatment with practolol following recovery from acute myocardial infarction) Seventy-one hospital centres participated and over 3 000 patients were randomly allocated to practolol (200 mg b d.) or placebo and were treated for 1 3 years. The final results are being analysed now and are not yet available However it is possible to present the interim results discussed at a symposium of participants held in London in October 1974

There was a significant difference between drug and placebo groups in relation to overall cardiac mortality (45 v 65 p <0 03 single-side test) and sudden deaths (i.e. occurring within hours of the onset of symptoms of a new event) (21 v 45 p <0 01)

The apparent reduction in mortality was especially evident in patients who had sustained an original pre-trial infarct in the anterior position (20 v 47 p <0 01)

Patients with original inferior infarction showed a reduction in sudden deaths but no overall reduction in the number of deaths

Because of the severe ocular and peritoneal re actions reported elsewhere with practolol, the drug is now considered to be unsatisfactory for long-term use However the good results could almost cer tainly be obtained using other beta-blocking agents. Professor Werko s group (Wilhelmsson, C *et al* Lancet 2 1157 1974) and Ahlmark *et al* (Ahlmark G *et al* Lancet 2, Corresp 1563 1974) have already published promising results using alprenolol The work of Wolfson and Amsterdam (Amsterdam Wolfson Gorfin, Ann. Int. Med 68/5 1151 1968) and Lambert (Lambert, D.N.D Lancet 1 972, 1972 Lambert D N.D Brit. Med. J 3 685 1974) suggests that propranolol and other beta-blockers may save life in the long-term treatment of angina pectoris and hypertension. It is therefore recommended that a beta-adrenoceptor blocking agent other than practolol should be used in the long term prophylactic treatment of patients recovered from anterior infarction.

Dr Werkö

We did a similar trial in a much smaller group of patients with myocardial infarction and followed them for two years. It was a double-blind trial with the patients receiving alprenolol twice daily for two years and compared to placebo We had the same results – a large reduction in the incidence of sud-

den death, enough to also give a reduction of total death in the treated group as compared to the placebo group We did not find any reduction in the number of new events of myocardial infarction in that trial. There were ..30 patients altogether in that trial. It was published in the Lancet in November last year (Wilhelmsson *et al.*, Lancet 1157 1159 1974).

Dr Green

I am glad that you did that trial because it confirms our own. With regard to non-fatal reinfarctions we found a reduction but the figures did not quite reach statistical significance I feel sure they would have done if we had continued the trial. In view of your own results, and promising evidence of life saving with propranolol in angina pectoris and hypertension, it seems very likely that other beta-blockers could be used satisfactorily for long-term prophylactic treatment in post-infarction.

Dr Braunwald

What about the incidence of heart failure in patients who developed reinfarction?

Dr Green

There was no significant difference between groups in relation to congestive failure Patients who developed heart failure after a new infarct were usually removed at once from the trial and the number of withdrawals because of heart failure was slightly higher in the drug treated group but not significantly so The numbers of deaths in patients after withdrawal from the trial were almost exactly the same in the two groups.

PRACTOLOL IN ACUTE MYOCARDIAL INFARCTION

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SUMMARY

A double blind trial of practolol in coronary heart disease has been conducted for 2 years

In 798 patients with acute myocardial infarction there was no reduction in overall mortality In a group with initial heart rate over 100 per minute mortality was significantly lowered up to 1 year

Of 484 patients with coronary heart disease treatment for years did not produce a significant reduction in infarction or sudden death

Beta-adrenergic blocking drugs have been shown to reduce left ventricular work and to have an antiarrhythmic action. On these grounds they would seem theoretically to have a place in the management of acute myocardial infarction. Practolol is cardio-selective beta-blocking agent with an intrinsic sympathomimetic action, but devoid of local anaesthetic effect. It has been found effective in post infarction arrhythmias (1) In early infarction it reduces the area of necrosis as measured by surface ST segment mapping (2).

METHODS

We have carried out a randomized double blind trial of 500 consecutive patients admitted to a coronary care unit with suspected acute myocardial infarction. Three quarters were admitted by a mobile coronary care unit. Patients were given either 300 mg practolol 12 hourly or an identical placebo. The initial dose was given as soon as the patient was seen and the tablets were continued for 2 years The majority of patients were monitored for at least 48 hours. We analysed the results at the end of 3 months and again at 2 years.

Two hundred and ninety-eight of the patients had undoubted acute myocardial infarction as judged by clinical history serial electro-cardiograms and serum enzymes. One hundred and eighty-three of these patients were male i.e. 61% The mean age of the patients was 64 years and ranged from 32 to 87 years The coronary prognostic index (C P.I) as devised by Norris *et al*(3) was calculated for each patient We have treated this as an estimate of the severity of the infarct The mean coronary prognostic index was 5 units and ranged from less than to greater than 18 The interval between the onset of the attack and coming under intensive care conditions varied from 7 minutes to several days but half the patients were seen within 3 hours 15 minutes One hundred and fifty-one patients i e 51 % received practolol and the remainder placebo. There was no significant difference between the two groups in any of the factors examined above – age sex coronary prognostic index or delay time (Table 1)

RESULTS AFTER YEARS

In Tables 2 and 3 we have summarized some of our results. We considered the mortality ventricular irritability bradycardia and heart failure. Mortality at 3 months on practolol was 15 % compared to 17 % on placebo while at 2 years the figures were 27 % and 31 % respectively Ventricular irritability as measured by the use of lignocaine was also similar The figures given refer to the second 8 hour period of monitoring when lignocaine usage was maximal but no difference in the two groups was noted at any time In the second 8 hour period 56 patients (37 %) on drug required lignocaine compared with 48 patients (33 %) on placebo Incidence of individual ventricular rhythm disorders was also similar

Table 1 All patients with acute myocardial infarction.

	Practolol	Placebo
Number of patients	151 51 %	147 49 %
Male sex	96 64 %	87 59 %
Mean age	62 years	63 years
Mean coronary prognostic index	5.8	5.6
Delay time under 3 hours	50 %	47 %

Table 2. All patients with acute myocardial infarction.

	Practolol		Placebo	
Mortality at 3 months	23 patients	15 %	25 patients	17 %
Mortality at 1 year	30 patients	20 %	37 patients	25 %
Mortality at 2 years	41 patients	27 %	46 patients	31 %

Bradycardia with a rate less than 60 per minute due to sinus bradycardia junctional bradycardia, or heart block was initially treated by atropine. In the first 8 hours of monitoring 49 patients on practolol (32 %) needed atropine compared with 28 patients (19 %) of those on placebo The difference is significant and an increased requirement for atropine was also noted in the second and third 8 hour periods

Clinical heart failure was said to be present when 3 of the following – basal crepitations, a third heart sound, a venous pressure greater than 3 cm above the sternal angle or x-ray evidence of left ventricular failure – were noted In the first 8 hour period failure was present in 49 patients (3[illegible] %) of those on practolol and *only* [illegible]9 *patients (20 %) on placebo* The higher incidence of heart failure was no longer apparent between 24 hours and two years

Since practolol did not influence the outcome of the overall group of patients we looked more closely at a sub-group who might be expected to have a high sympathetic dri e and hence obtain greater benefit. Fifty-three patients, when initially seen had a heart rate of more than 100 beats per minute Twenty-three of these received practolol and the remaining 30 the placebo Fifteen patients (65 %) on drug and 13 patients (43 %) on placebo were male The age of patients on the drug ranged from 48 – 78 years with a mean of 67 years. In the placebo group mean age was 64 years and ranged from 48 – 82 years The mean coronary prognostic index for the two groups was similar 7 4 units for patients on drug 7.2 units for the remainder

Median delay time was rather shorter in the practolol group 2 hours 50 minutes compared with 3 hours 52 minutes for those on placebo The initial rhythm of 15 patients (65 %) on drug and 20 (67 %) of those on placebo was sinus tachycardia. The remaining patients were either in atrial fibrillation or supraventricular tachycardia.

Ten patients (43 %) on the drug required lignocaine for ventricular irritability during the first 48 hours compared with 15 patients (50 %) of those receiving placebo The requirement of atropine was predictably low 3 patients on the drug and 2 on placebo received atropine.

The incidence of the following rhythm changes during monitoring – ventricular tachycardia, multifocal ectopics, R on T ectopics and ventricular ectopics occurring more frequently than 100 per hour – was examined and no significant difference was noted between the two groups.

Cumulative mortality is shown in Table 4 After 24 hours no patient on practolol had died while 4 in the placebo group were dead. At the time of discharge from hospital 1 patient (4 %) had died in the practolol group compared to 7 patients (23 %) in the placebo group At 3 months mortality in the placebo group had risen to 33 % ($p < 0.05$) The difference between the two groups was also significant at one year but not at two

In summary when we look at all patients with acute myocardial infarction we find in association with practolol

1 no change in mortality
2 heart failure in the first day was more common
3 incidence of bradycardia higher on the first day

In patients with a rapid initial heart rate we found practolol associated with

1 reduced mortality up to 1 year
2. minimal requirement of atropine in the acute phase

These findings suggest that beta-blockade may be beneficial in patients with acute myocardial infarction with a high heart rate when first seen.

Table 3 All patients with acut myocardial infarction.

	Practolol		Placebo	
Ventricular irritability Lignocaine use second 8 hours monitoring	56 patients	37 %	48 patients	33 %
Bradycardia Atropine use first 8 hours monitoring	49 patients	3 %	28 patients	19 %
Clinical heart failure First 8 hours monitoring	49 patients	32 %	29 patients	20 %
Clinical heart failure Three month follow-up	9 patients	7 %	17 patient	14 %

MYOCARDIAL INFARCTION AND SUDDEN DEATH AFTER DISCHARGE FROM HOSPITAL

In addition to the 298 patients with myocardial infarction there were 186 with prolonged cardiac pain but without evidence of infarction These 484 cases were followed for 2 years.

During the follow-up period there were 44 episodes of acute infarction or sudden death Of these 27 were receiving placebo and 17 practolol. During the trial there were 2730 patient-months on practolol and 3814 patient months on placebo giving an incidence of infarction 5 4/1000 patient-months with placebo compared with 3 4/1000 patient months with practolol The difference does not attain significance

The patients receiving practolol at the time of infarction were clinically similar to those receiving placebo Age sex, coronary prognostic index, heart rate and blood pressure are shown in Table 5

Patients with sinus bradycardia of less than 60 per minute associated with hypotension or ventricular irritability were treated with atropine or pacing as were those with Mobitz type 2 or complete heart block. The incidence was similar in the groups – 26 % of those on placebo 29 % of those receiving practolol.

Patients with ventricular irritability i.e. those with ventricular fibrillation, ventricular tachycardia or with frequent, multifocal consecutive or R on T ectopic beats, were treated with lignocaine or similar drugs. This was required in 41 % of those on placebo and 29 % of those on practolol This difference is not significant.

X-ray evidence of alveolar or interstitial pulmonary oedema was seen in 35 % of patients on practolol and in 41 % on the placebo

Mortality in the 2 groups was high but similar – 52 % of those on placebo within 2 weeks of onset compared with 53 % of those on practolol The high mortality reflects the inclusion of all deaths and not just those in hospital There were 2 deaths in the treated series within 4 hours as against 6 in those on the placebo (Table 6). This trend is in accord with the findings of Wilhelmsson *et al* (4).

Table 4. Patients with initial heart rat over 100/minute cumulative mortality

	Drug		Placebo	
First 24 hours	0		4	13 %
At hospital discharge	1	4 %	7	23 %
At 3 months	1	4 %	10	33 %
At 1 year	2	9 %	13	43 %
At 2 years	5	22 %	14	47 %

Table 5 Patients receiving practolol at time of infarction

	Practolol	Placebo
Age	65 ± 9	59 ± 14
Sex Male	13	19
Sex Female	4	8
C P.I	9 1 ± 5 1 (15 patients)	8.6 ± 4.8 (23 patients)
Mean Heart rate	88.3 ± 18 7	85.5 ± 23 4
Systolic B P	118 ± 40	118 ± 32

ADVERSE EFFECTS

Seventeen patients had stopped the tablets because of adverse side effects by 3 months of these 3 were on placebo The most troublesome were constipation and skin rashes 2 of which were psoriaform. There were no eye complications nor did we encounter sclerosing peritonitis (5)

Antinuclear factor was sought after 1 year in 66 random patients, 35 taking practolol 31 on placebo It occurred in 12 of those on the drug and in 3 on placebo ($p < 0.05$)

DISCUSSION

Practolol as a beta-blocking agent, might be expected to prevent arrhythmias due to excessive sympathetic drive Experimental evidence suggests this effect would be maximum in the very early phase of infarction when the patient is rarely under close medical supervision (6) Patients already on practolol might suffer less from early arrhythmic death Fox *et al* (7) have suggested that beta-blockade may protect some patients from the development of myocardial infarction and Fischl *et al* (8) have shown its value in acute coronary insufficiency

The incidence of infarction in our study was lower in those receiving practolol but the difference might have occurred by chance. The incidence of death within 24 hours was also lower in patients taking the drug (Table 6) but this difference disappeared at 2 weeks. The requirement of anti-arrhythmic drugs for ventricular irritability was lower in patients receiving practolol but again the differ

Table 6. Time of death

	Practolol	Placebo
Within 1 hour	1 (6 %)	1 (4 %)
1 – 24 hours	1 (6 %)	5 (19 %)
24 hours – 2 weeks	7 (41 %)	8 (30 %)
Total	9 (53 %)	14 (52 %)

ence may have been by chance Beta blockade may precipitate heart failure in patients with a damaged myocardium However in this study mortality in the 2 groups was similar and there was no evidence that practolol produced heart failure Other workers have published similar results (7).

The tablets were supplied to patients at 4 weekly intervals and we believe they were taken. Initial pulse rate and blood pressure (Table 5) were similar in the 2 groups This was also the finding of Fox *et al* (7)

If the reduction in the incidence of myocardial infarction and of ventricular arrhythmia noted here is not due to chance the size of trial necessary for statistical confirmation would be about 2000 patients followed over 2 years In practice this would need to be done as a multicentre trial

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DISCUSSION

Dr Fitzgerald

Dr Barber I have two questions Firstly in the light of Dr Green s results did you compare the prognosis in patients having anterior infarctions with those having posterior infarcts Secondly do you have any information regarding patient compliance with therapy? I think that when prospective studies are being planned the question of patient compliance is absolutely vital

Dr Barber

Yes I would entirely agree Firstly we do not have information regarding splitting them into anterior and inferior infarcts. We did serum practolol levels in the last 100 patients and they were all in the therapeutic range The other thing we looked at which has nothing to do with your question was antinuclear factor In 66 random patients we found it in 32 per cent of those on practolol and in 10 per cent of those on placebo None of them had disseminated lupus Reviewing figures there was no reduction of mortality within any subgroup of site of infarct

Dr Johansson

In your subgroup comprising patients with more than 100 beats/min there was an initial reduction of mortality If you exclude that initial reduction did you have an additional significant mortality decrease during the two year follow-up?

Dr Barber

It is significant up to one year but it i not significant up to two

Dr Johansson

Even if you exclude the short term decrease during the acute phase of the infarction

Dr Br unwald

If you start the patients off after they have been discharged from the hospital and if you exclude the

obviously initially beneficial effects is there a further difference between the two groups?

Dr Barber

I do not think we know that. Can you answer that, Dr Boyle

Dr Boyle

The difference is not significant if you exclude the initial mortality

Dr Braunwald

Can you tell us the causes of death in the patients in that group with heart rates over 100/min who died – not the practolol group but the placebo group In other words – was the excess of death due to cardiogenic shock, pump failure

Dr Barber

The plasma practolol levels were measured three times during the first four days and then at discharge from hospital and at three months follow-up And as I say perhaps to our surprise, they all seemed to be taking practolol Cardiogenic shock or heart failure is the most common cause of death

Dr Braunwald

Which group is that

Dr Barber

These are the 44 patients who died while taking practolol.

Dr Braunwald

The group that I am interested in is the one with the initial tachycardia, that subgroup that was on placebo. In other words, what deaths did the practolol group appear to prevent?

Dr Barber

I do not think I can answer that.

Dr Braunwald

But those data are available

Dr Barber

Yes I could get that

Dr Braunwald

The reason why I am so fascinated by this is that it is going to take a long time to be able to get this kind of information If beta-blockade plays a role in protecting patients in terms of infarct size rather an antiarrhythmic effect, then it is going to be most potent when it is administered as early as in your unit So this is really a very unique population Also what is interesting about this is that you have selected out that subsample which might most logically be helped if this proposed mechanism is operative Therefore, it would be extremely interesting to see if those deaths in the placebo group are actually due to heart failure

Dr Boyle

I think that if we did look at them we would find that the majority of them would be in heart failure or shock In criticism of our own paper we started with an oral dose so in fact quite a lot of the advantage that we should have got by seeing our patients early was lost It might have been some time before a therapeutic level was reached. It would have been a better study if the initial dose had been given intravenously Our present policy with patients we see early with sinus tachycardia is to use a beta-blocker intravenously Reviewing the figures the incidences of death from heart failure and shock over the two year period in patients with an initial heart rate greater than 100/min was significantly lower (4 per cent in patients given practolol and in those on placebo 29 per cent) The difference is significant at the 0.01 level

Dr Braunwald

How long is it before you get any physiological effect – the lowering of heart rate

Dr Boyle

We start getting it within an hour

Dr Braunwald

Well, you are still ahead of most of us by a couple of hours which may be a very crucial time.

Dr Boyle

At present we use either practolol or sotalol Recently we have been using sotalol more We have found that in patients with sinus tachycardia practolol reduces heart rate less than sotalol. The difference is maybe 10 beats per minute

Dr Fitzgerald

Could I comment on this because I certainly have a very healthy respect as I am sure you have for the intravenous use of beta-blocking agents in acute myocardial infarction. I wonder whether one may not lose some patients because certain doctors who are not familiar with the intravenous use of beta-blockers may give an inappropriate dose for the possible gain of half an hour Practolol as we have shown, is well absorbed and effective blood levels are obtained within 40 minutes On the other hand it is possible that in the acute infarct situation the absorption is delayed.

Dr Barber

Another side to this work is could we evolve a combination of drugs which could be given routinely by the family doctor? You know Pantridge s work on this and there is a great deal of other work which still goes on It is proving difficult to achieve I suppose that is what you would expect. We have tried atropine and sotalol or practolol but I would entirely agree with Fitzgerald that at this moment we would not suggest a routine use of beta-blockers in the acute situation. It is quite a different matter when a coronary team does it on a strict protocol As soon as they have got the patient painfree and stable there is no hurry It is feasible for them to wait 30 or 40 minutes if necessary

Dr Braunwald

Did you see the striking relief of pain that was reported earlier?

Dr Barber

We have seen this, but we have been a little disappointed Sometimes we get a gratifying result but sometimes not so

Dr Braunwald

But your administrations were intravenous

Dr Barber

Again we have done this in a limited number of patients

Dr Braunwald

Did you have some comments Dr Waagstein?

Dr Waagstein

I was interested in the group with tachycardia. How many in this group with tachycardia had decompensation?

Dr Barber

Yes the most probable explanation is that the sinus tachycardia was a manifestation of the left ventricular failure

Dr Waagstein

Usually we use tachycardia as a sign of left ventricular failure particularly impending failure.

There is another question about the patients. You divide them, I think, into those with and without decompensation. Is it right that most of the patients were included in the study and very few were excluded due to decompensation?

Dr Barber

That is correct. We admitted them to the study We treated their heart failure but we admitted them to the trial.

Dr Waagstein

I would like to see the group which was decompensated from the beginning. How was the mortality in that group compared to the group which was not decompensated at all? Was there any difference between those two groups?

Dr Boyle

We were very liberal in admitting patients to the trial The only patients who did not get an oral dose were those who were moribund when first seen Clinical heart failure was not a contraindication. We thought it would be safe to use practolol with diuretic treatment. I certainly can find no evidence that we did any harm On chest x-ray rather than clinical grounds we found no evi-

dence of increased heart failure That is based on an x-ray taken within 24 hours.

Dr Barber

This is correct.

Dr Boyle

Could I mention just one other point. One of the advantages of using a beta-blocking drug in the patient s house is that the change in heart rate when the patient is moved is avoided. Mulholland and Partridge have shown that marked tachycardia can occur when patients are moved from home to ambulance and from ambulance to hospital. This is largely prevented by prior injection of beta-blocking drugs. If a family doctor were to give the drug by mouth, as Professor Fitzgerald suggested, he would have to wait some 40 minutes before the patient could be moved. Presumably he would not have the benefits of monitoring at this time and he would be looking after the patient at the most hazardous phase. There is therefore a case for giving the drug intravenously and I feel practolol may be safer than sotalol

Dr Braunwald

There have been two papers in the past year on large dose of xylocaine (lignocaine) at the time the patient with acute myocardial infarction is first seen at home Would you comment on this Which drug would you prefer? I gather you prefer the beta-blocker but I wonder if you would comment on the potential difference

Dr Boyle

There is now a lot of evidence particularly in the early hours of myocardial *infarction* that many ventricular arrhythmias are resistant to lignocaine. Patients with a normal heart rate early in the infarct seem to respond well to lignocaine. Those who have sinus tachycardia respond poorly In Belfast we tend to use a beta-blocker to slow the heart rate and if ectopics still persist at this stage we then use lignocaine Our impression is that if you can slow the heart rate you improve the response to lignocaine. It could be that if we need a magic mixture it should contain a beta-blocking drug to slow the heart rate and perhaps atropine to prevent too much slowing, and lignocaine to deal with ectopic beats and perhaps there is something else too, say hyaluronidase which was discussed earlier

Dr Hjalmarson

It might be of some value if Dr Barber and his group could exclude the group you needed to treat before they could go into the trial or those who had clear decompensation when they started I do not think they should be in a beta-blocker study First one should make a selection and exclude patients who are likely to go into heart failure those who already have pulmonary edema. On the other hand we have already treated 5 patients with acute myocardial infarction who had pulmonary edema and a high heart rate with beta-blocker The pulmonary edema disappeared when the heart rate was reduced. I think it is important with the i.v dose of beta-blockers in patients with acute myocardial infarction not just the fast effect but to get off the effect fast too I mean, if you inject an i dose you will be off that beta-blockade in about 60 minutes If that patient should develop heart failure there will be a problem during that short time while if you give him an adequate oral drug like practolol you might have a problem for 12 or 24 hours especially in a dose of 300 mg twice daily An important information from Dr Barber s study is that the patients tolerated beta-blockade so well. That means that beta-blockade is safe even in acute myocardial infarction. In the trial in Göteborg we start with an i v dose of beta-blockers to see if the patient will tolerate it. Then we start an oral therapy after 60 minutes We believe that if you have an ischemic part of the myocardium you test the function of the rest of the heart while on beta-blockade. It is not likely that there will be problems with heart failure later on if i.v beta-blockade was tolerated early I do not think it matters that the anterior wall, which may have been ischemic early will be necrotic later That ischemic area of the heart will not be functioning either

Dr Barber

Thank you very much I would accept your remarks As my colleague Dr Boyle said once you start a trial you want to alter the design immediately and we have had all sorts of other thoughts about this trial which we started more than two years ago.

Dr Braunwald

Well I think it has been most instructive and an extremely interesting paper

THE IMPORTANCE OF MECHANICAL PERFORMANCE FOR DEVELOPMENT OF MYOCARDIAL INFARCTION IN MAN

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SUMMARY

In studies using an experimental infarction as a model it has been shown that factors increasing myocardial oxygen consumption will increase the size of infarction, while factors reducing the oxygen consumption have the opposite effect. The presence of catecholamines might be most important. It is suggested that local release of noradrenaline from the sympathetic nerve endings in ischemic myocardium can induce vigorous contractions and deleterious ischemia and result in cellular necrosis.

A retrospective study was performed in 81 patients hospitalized in Göteborg in 1964-1965 due to attacks of severe chest pain with no previous documented myocardial infarction. In 31 of these patients definite congestive heart failure was seen at hospitalization or developed later. During 10 years of follow up the mortality was 48 per cent and an acute myocardial infarction was found in 64 per cent of the patients without congestive heart failure. In patients with congestive heart failure the mortality was 42 per cent, and 6.4 per cent had an acute myocardial infarction. The poor mechanical performance and a lower myocardial content of noradrenaline of the failing heart might protect from the acute myocardial infarction and instead be predisposed to a slow degeneration, pump failure and serious arrhythmias.

Severe angina pectoris and congestive heart failure might represent opposite ends of the spectrum of ischemic heart disease with similar degrees of luminal narrowing of the coronary arteries.

INTRODUCTION

A number of studies mainly from Braunwald and co-workers (Maroko *et al.* 1971, 1973, Reimer *et al.* 1973, Braunwald & Maroko, 1974) have shown that factors increasing the heart work and thus the myocardial oxygen consumption will increase the severity and extent of myocardial injury following an acute coronary occlusion in dogs. Factors reducing heart work and oxygen consumption have the opposite effect. If this holds true in man, the therapy in the coronary care unit should be focused on reduction of heart work and on optimal coronary flow (Table I). It is most likely that accumulation of metabolites in the ischemic area is the direct cause of cellular damage (Neely *et al.*, 1973, 1975). Maintenance of venous drainage and diffusion of metabolites from the ischemic area might be more critical than supply of oxygen and substrates. The enhancement of diffusion of metabolites from the ischemic zone by hyaluronidase and the stabilization of lysosomal membranes, suggested to be obtained by e.g. methylprednisolone, could be favourable interventions to protect the jeopardized myocardium (Spath *et al.* 1974, Maroko *et al.* 1975).

In general terms, myocardial ischemia is due to an imbalance between myocardial metabolic de-

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Table I. Therapy for reduction of myocardial ischemia and infarction size

A. Reduction of heart work
- β-blockade
- Vasodilatation (α-blockade, nitroglycerin)
- Reduction systolic blood pressure
- Reduction heart rate
- Reduction venous return (Lasix, pethidine)
- Heart failure treatment
- Arrhythmia treatment

B. Optimal coronary flow
- Prevent hypotension
- Reduction heart rate

C. Enhancement of diffusion in ischemic zone
- Hyaluronidase

D. Stabilization of lysosomes
- Corticosteroids

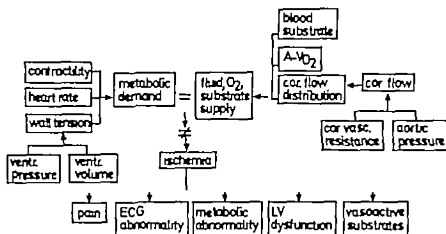


Fig. 1 Factors of importance for development of ischemia.

mand and coronary artery supply of fluid oxygen, and substrates (Fig. 1) It does not matter whether the development of coronary artery thrombosis is a primary event or is secondary to severe ischemia (Chandler *et al* 1974 Erhardt, 1974). Optimal coronary flow and minimal heart work should be obtained. In this respect, adrenergic beta-blocking agents are of value and the positive effect of beta-blockade on myocardial ischemia is well documented in a large number of controlled trials in patients with angina pectoris (Table II). Side-effects of beta-blockade in these patients are few How ever every clinician keeps in mind the possibility of inducing congestive heart failure in some of these patients with poor heart function. Congestive heart failure and cardiac enlargement have been considered to be associated with increased mortality in patients with angina pectoris (Block *et al* 1952 Weinblatt *et al* 1968 Kannel & Feinleib 1972 Reeves *et al* 1974). Congestive heart failure has also been suggested a risk factor for development of an acute myocardial infarction (e g Weinblatt *et* al 1968 Braunwald & Maroko 1974) Contrary to this it has been found that isolated rat hearts could avoid severe ischemia and development of myocardial infarction following a reduction of coronary flow if the hearts got pump failure with reduced heart rate and pressure development (Neely & Morgan Waldenström & Hjalmarson, unpublished observations)

Table II β-blocking agents in angina pectoris – a summary of controlled trials which were published before January 1975

No. of controlled trials	No. of patients	Trials with beneficial effect of β-blocker	Trials with no beneficial effect
56	1 314	53	3

Criteria. Effort tolerance
Nitroglycerin consumption
Attacks of anginal pain

Based chiefly on alprenolol, oxprenolol practolol, and propranolol.

The present study was undertaken to find out whether definite congestive heart failure is a risk factor for development of acute myocardial infarction in patients with angina pectoris. A retrospective study was done in which patients having angina pectoris were compared to patients with angina pectoris and congestive heart failure The patients were hospitalized in Göteborg in 1964-1965 due to attacks of severe chest pain and there are 10 years of follow up

METHODS AND MATERIALS

A total of 81 patients were hospitalized in Göteborg in 1964-1965 due to attacks of severe chest pain at rest given the diagnosis angina pectoris The patient material is shown in Table III as well as the definitions of angina pectoris and congestive heart failure Congestive heart failure was present at hospitalization or developed later Patients with evidence of valvular disease or definite or probable acute myocardial infarction, as judged from history or ECG were excluded. The material included 50 patients classified as angina pectoris and 31 patients classified as angina pectoris and congestive heart failure (Table IV) The angina pectoris was of Class IV according to New York Heart Association. Comparing the two groups more females a larger heart volume more hypertension and atrial fibrillation as well as therapy of digitalis and diuretics were found in patients with congestive heart failure In the patients considered as having no definite congestive heart failure 34 per cent were on digitalis therapy

Table III. A ten year retrospective follow up of 81 patients with angina pectoris hospitalized in 1964-1965. Patient material and definitions of angina pectoris and congestive heart failure.

Anginal pain and no congestive heart failure*	50 patients
Anginal pain and clinical signs of congestive heart failure**	31 patients

* Clear effort anginal pain
Regular nitroglycerin consumption
Physical work capacity limited by pain
No previous myocardial infarction

** Dyspnea also without chest pain and/or peripheral edema.
Favourable effect of digitalis and/or diuretics

Table IV

	Angina pectoris	(%)	Angina pectoris + congestive heart failure	(%)
Patients	50		31	
Ages	57 ±1.3		59 ± 1.4	
Males	44	(88)	19	(61)
Females	6	(12)	12	(39)
Years of angina pectoris until 1965	5.4±0.8		4.9± 0.9	
Heart volume ml/sq.m.	386 ±8		459 ±21	
Hypertension (>110 diastolic)	20	(40)	21	(68)
Atrial fibrillation	1	(2)	10	(32)
Digitalis	17	(34)	26	(84)
Diuretics	13	(26)	23	(74)

Description of the two groups of patients classified as angina pectoris and angina pectoris + congestive heart failure. Fatal acute myocardial infarction (AMI) includes patients who died within three weeks after the infarction. Late post-AMI deaths include patients who died more than three weeks following their infarction due to sudden death, reinfarction or pump failure. Sudden death is defined as death within one hour after onset of symptoms as chest pain or arrhythmia and no myocardial infarction or any other cause of death was found at autopsy. Cardiogenic shock includes patients who died in the picture of pump failure with no myocardial infarction found at autopsy.

and 26 per cent were given diuretics without any clear indication for such treatment and no symptoms of heart failure.

A coronary angiogram was obtained in 11 patients without and in 4 patients with congestive heart failure. Autopsy was performed in 92 per cent of the 37 patients who died from 1965 to 1975. A marked luminal narrowing of the coronary arteries was described in most patients and no difference was seen between the two groups. Very small parts of old scar tissue were accepted in patients classified as having had no episode of acute myocardial infarction, while those who had a larger well defined area of scar tissue were excluded. The diagnosis acute myocardial infarction required two of the three clinical criteria: 1) severe chest pain or acute heart failure, 2) typical ECG series, and 3) diagnostic elevation of serum enzymes indicating muscle necrosis or a fresh myocardial infarction at autopsy. All hospital records were reviewed. Sudden cardiac death was diagnosed when death occurred under conditions suggestive of a heart attack within one hour after onset of symptoms and when no other explanation for the death was available. Cardiogenic shock includes patients who had no signs of acute myocardial infarction but who developed either a severe backward failure with pulmonary edema or general edema or forward failure with hypotension and shock and died under these circumstances. Early and late myocardial infarction deaths include arrhythmias and cardiogenic shock.

RESULTS

During the course of 10 years after hospitalization for severe angina pectoris 48 per cent of 50 patients with no congestive heart failure died compared to 42 per cent of the 31 patients who had congestive heart failure (Table V). There is no statistical difference in total mortality. Patients without failure died more often in relation to their acute myocardial infarction (26 per cent died within two weeks after the infarction) or following the infarction (16 per cent died later than two weeks after the acute myocardial infarction) while the patients with failure died more suddenly (10 per cent) or in cardiogenic shock (19 per cent). There was no clear difference in non-cardiovascular deaths.

Comparing patients in the two groups who were alive after 10 years of follow up dyspnea and peripheral edema were the main symptoms in the patients with congestive heart failure while anginal pain was the most common complaint among the patients in the non-failing group. Table VI shows all survivors including 11 patients in the non-failing group who had acute myocardial infarction during the follow up. No patients with myocardial infarction in the failing group were alive after 10 years. In patients with more marked signs of congestive heart failure their chest pain often disappeared during the follow up. Most patients in the failing group reported their anginal pain much less severe than it was earlier in the period. In the

non-failing group anginal pain disappeared in some patients who did not have symptoms of dyspnea or leg edema. In this group of non-failing patients 6 patients (23 per cent) had dyspnea and 3 patients (11 per cent) had leg edema. All these patients had a previous myocardial infarction during the period of follow up

DISCUSSION

From this study it is suggested that development of congestive heart failure might protect the heart from the acute episode of myocardial infarction and instead result in a degeneration of the myocardium similar to the aging process with a slow and proportional reduction of mechanical performance of the heart and in coronary flow. This is in accordance with the observations by Roberts and co-workers (1974) from autopsy of patients with severe angina pectoris and congestive heart failure. Histologic examination of the coronary arteries showed no differences in degrees of luminal narrowing between those who died suddenly after severe angina pectoris and those who died under conditions of congestive heart failure. 'Severe degrees of either angina pectoris or chronic heart failure rarely occur in the same patient. Angina pectoris and congestive heart failure were suggested to represent opposite ends of the spectrum of ischemic heart disease. Generally congestive heart failure is considered to be associated with poor prognosis with respect to both development of an acute myocardial infarction and probability of death (Block *et al* 1952 Weinblatt *al* 1968 Reeves *et al* 1974 Russek, 1974). However the congestive heart failure in these reports is not definite (Weinblatt *et al* 1968) or is

Table V

	Angina pectoris		Angina pectoris + congestive heart failure	
	N	(%)	No.	(%)
Patient	50		31	
All AMI	32	(64)	2	(6 4)
Fatal AMI	13	(26)	1	(3.2)
Nonfatal AMI	19	(38)	1	(3)
Sudden death	0		3	(9 7)
Cardiogenic shock	0		6	(19)
Noncardiovascular deaths	3	(6)	3	(9 7)
Late post-AMI deaths	8	(16)	0	
All deaths	4	(48)	13	(4?)
Survivors 10 years	?6	(52)	18	(58)

Table VI

	Angina pectoris		Congestive heart failure + angina pectoris	
	No	(%)	No	(%)
Alive after 10 years.	26		18	
Symptoms. Dyspnea	6	(23)	18	(100)
Dyspnea + leg edema	3	(11)	11	(61)
Anginal pain	18	(69)	11	(61)
Hypertension	10	(38)	14	(78)
Atrial fibrillation	1	(38)	6	(33)
Digitalis	6	(23)	15	(83)
Diuretics	5	(19)	11	(61)

Reduced pain

Symptoms, observations, and treatment in the patients who were alive after 10 years. In the group of angina pectoris 10 patient had a myocardial infarction and all patients with dyspnea or leg edema developed these symptoms after an infarction. I the group of angina pectoris and congestive heart failure none was alive of those who had myocardial infarction. In both groups there was reduction in number of patients with anginal pai and this was most obvious in the group of patients with congestive heart failure Those with more marked symptoms of heart failure had less severe chest pain compared to the others.

mostly secondary to an acute myocardial infarction (Block *et al* 1952 Reeves et al. 1974 Russek, 1974). It is possible that some people have a more aggressive form of ischemic heart disease and are more likely to develop an acute myocardial infarction. A clinical history of a previous myocardial infarction will in that case mean a poor prognosis with respect to reinfarction and death (Vedin *et al* to be published)

In this study it was observed that in patients who developed marked symptoms of congestive heart failure with dyspnea and leg edema angina pectoris often disappeared. The disappearance of angina pectoris after it has been present for years has been attributed to the development of collateral circulation or to the replacement of the ischemic myocardium by scar tissue after a myocardial infarction (Friedberg, 1966) In the Framingham study angina pectoris does not disappear more after a myocardial infarction than it does spontaneously without infarction (Kannel & Feinleib 197?). In the study of Kannel and Feinleib it is mentioned that loss of angina pectoris is reported to occur after onset of congestive heart failure and atrial fibrillation, which seems to be an old unpublished observation among clinicians. This is in agreement with the findings in the present study It is recognized that angina of *recent onset or angina which has changed in severity* has a less beneficial prognosis compared to a

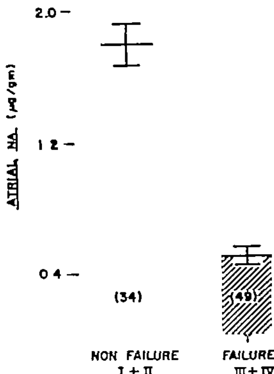


Fig. 2. Concentration of noradrenaline (NA) in atrial appendage biopsies taken during cardiac operations from 34 patients without heart failure (non-failure Classes I + II) and 49 patients with heart failure (failure Classes III + IV). The average values and their standard errors are shown. (Reproduced by permission of Proc. R. Soc. Med 58. 1063-1066, 1965.)

more constant angina which has continued for years The unstable angina may be premonitory to a myocardial infarction or sudden death (Friedberg, 1966 Harrison & Reeves, 1968) In the Framingham study only 23 per cent of infarctions were preceded by angina (Kannel & Feinleib 1972) indicating a sudden development of severe ischemia in an area of the myocardium.

It has been reported that youth may predispose to sudden death in coronary attacks although elderly people in general have more advanced coronary atherosclerosis (Kannel *et al* 1975). This might reflect a lower response of the aged myocardium to locally released noradrenaline (Lakatta *et al* 1975) rather than being explained by better developed collateral circulation (Kannel *et al* 1975). Cardiac enlargement and congestive heart failure are more *common at an advanced age. In the failing heart of* man and animal there is low noradrenaline content of both atrias and ventricles (Fig Braunwald & Chidsey 1965). The importance of stress and catecholamines for the development of angina pectoris and myocardial infarction was suggested many years ago (e g. Raab *et al* 1961 Raab 1962). There are at present a number of studies indicating a major role of catecholamines in the development of cellular necrosis and initiation of serious arrhythmias in the infarction process (e g Barrera *et al* 1966 Ceremuzyński *et al* 1969 Braunwald & Maroko 1974 Gavras *et al*., 1975 Waldenström & Hjalmarson 1975) A very early response to a certain degree of anoxia or ischemia in the myocardium is a pronounced release of noradrenaline (Fig. 3 4 Wollenberger *et al* 1967 Shahab *et al* 1972). Under anoxia there is an immediate cardiac arrest, while under ischemia the heart cells contract vigorously for a short period. The myocardial content of ATP is reduced to a lower level in ischemic myocardium compared to anoxic myocardium (Neely *et al* 1973) Furthermore after prolonged anoxic perfusion of isolated rat hearts in presence of glucose there are no signs of myocardial damage in contrast to the perfusion under ischemic conditions (Neely *et al* 1973 Hearse & Humphrey 1975) Cardiac arrest under anoxia might protect the heart from the toxic effects of locally released noradrenaline.

In studies of experimental myocardial infarction using a dog heart *in situ* Braunwald and co-workers (Braunwald & Maroko 1974) have shown that factors increasing heart rate or pressure development *will increase the size of infarction* (Table I) Using an isolated ischemic rat heart preparation this observation was confirmed in our laboratory (Waldenström & Hjalmarson 1975) The presence of catecholamines seems to be most important in both experimental models Long-term daily injections of adrenaline to rats reduced the myocardial content of noradrenaline and when made ischemic these

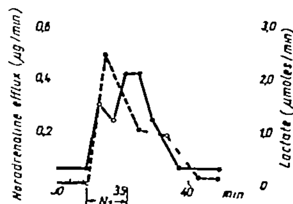


Fig. 3 Release of noradrenaline (solid line) and lactate (broken line) from an isolated perfused rabbit heart following anoxia by switch to anaerobic perfusion. (Reproduced by permission from Effect of Acute Ischaemia on Myocardial Function, Churchill Livingstone Edinburgh & London, 1972 (Eds. M. F Oliver D G Julian & K. W Donald), pp. 97 108).

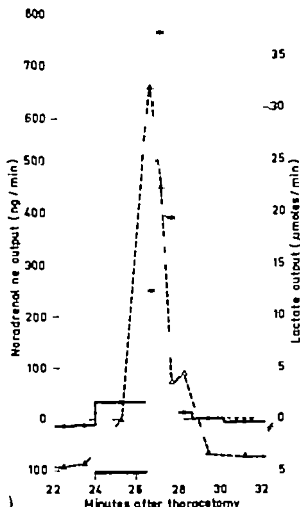


Fig. 4. Net output of noradrenaline (solid line) and lactate (broken line) from a portion of a dog's heart. The oblique bars on the abscissa denote the period of occlusion of the left anterior descending coronary artery. (Reproduced by permission from Coronary Circulation and Energetics on the Myocardium, S. Karger AG, Basel & New York, 1967 (Eds. G. Marchetti & B. Taccardi), p. 200).

hearts failed promptly but were not damaged as were the hearts of control-injected rats which did not fail as promptly (Waldenström & Hjalmarson 1975). It is obvious from experimental and clinical observations that ischemia can result in an acute myocardial infarction or in congestive chronic heart failure. A number of questions can be raised concerning these two clinical diseases as stated in Table VII. All these questions might be answered in the affirmative.

It is suggested that angina pectoris and congestive heart failure are clinically and pathophysiologically two diseases although both have similar degree of luminal narrowing of the coronary arteries. Furthermore both are due to imbalance between myocardial demand and supply

Table VII. Questions that can be raised concerning the two clinical syndromes: angina pectoris and congestive heart failure

Ischemic heart disease

1. Angina pectoris is one disease
   Congestive heart failure is another disease?
2. Both are ischemic heart diseases (IHD) with similar degree of luminal narrowing of the coronary arteries
3. Do they represent opposite ends of the clinical spectrum of IHD?
4. Are both due to imbalance between myocardial metabolic demand and substrate O_2, and fluid supply?
5. Will fast development of imbalance result in angina pectoris and acute myocardial infarction,
   and slow development of imbalance result in degeneration, fibrosis, and congestive heart failure?
6. Are the myocardial content and release of noradrenaline the key factors for angina pectoris and congestive heart failure?

of fluid (wash-out of metabolites), oxygen, and substrates. A fast development of imbalance will result in angina pectoris and myocardial infarction while a slow development of imbalance will result stepwise in hypertrophy, degeneration, fibrosis and congestive heart failure. Although other factors are certainly involved, the myocardial content of noradrenaline and the release of noradrenaline from sympathetic nerve endings are suggested to be most important in angina pectoris, acute myocardial infarction and congestive heart failure. In ischemic heart disease with severe chest pain the myocardial content of noradrenaline and the contractility are supposed to be well maintained while congestive heart failure has a low myocardial content of noradrenaline and reduced contractility (Fig. 5). It is not stated whether the reduced contractility is caused by the low myocardial content of noradrenaline or vice versa. As shown in Fig. 5 patients with angina pectoris or acute myocardial infarction will be threatened by the development of congestive heart failure. It would be less likely for a patient with marked congestive heart failure to develop severe angina pectoris or acute myocardial infarction.

It is suggested from the present paper that angina pectoris and congestive heart failure represent opposite ends of the spectrum of ischemic heart disease in agreement with others (Roberts *et al.* 1974). Furthermore a fast development of severe myocardial ischemia will result in angina pectoris and/or myocardial infarction while a slow development of myocardial ischemia will result in congestive heart failure. This is in accordance with observations from an ischemic rat heart preparation (Wal-

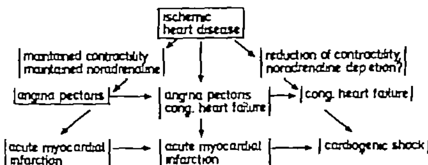


Fig. 5 Ischemic heart disease is due to an imbalance between myocardial metabolic demand and coronary supply of fluid, oxygen and substrates. A fast development of imbalance will result in angina pectoris or myocardial infarction, while slow development of imbalance will result in congestive heart failure. The general direction in the figure will be downwards or from left to right. Isolated severe angina pectoris and marked congestive heart failure represent opposite ends of the spectrum of ischemic heart disease

denström & Hjalmarson, 1975). The present study is retrospective and there could be small differences between the groups of patients in respect of severity of angina pectoris and degree of coronary atherosclerosis. A physical work test and a coronary angiogram were performed in only a few patients during their hospital stay in 1965 due to severe chest pain. To clarify the suggested mechanisms in this paper and to elucidate whether chronic congestive heart failure will protect from acute myocardial infarction rather than being associated with an elevated risk for infarction a prospective study has to be done under carefully controlled conditions.

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DISCUSSION

Dr Braunwald

This very provocati e paper i open for discussion.

Dr Morgan

I do not think I can really comment upon the clinical situation. It is a fact that the tolerance to ischemia is much better if the solated heart is allowed to fail by reduction in heart te than by eduction in pressure development. Whether or not this is important in patients I do not know but there s reasonableness to Dr Hjalmarson uggestions

Dr Fitzgerald

Do you have any views on the possible role of platelets in your scheme

D Hjalmarson

They might be involved I think it starts in most patients with some degree of atherosclerosis of the coronary arteries When getting a sudden imbalance between myocardial metabolic demand and supply of fluid substrate and oxygen there will be a release of norepinephrine from the sympathetic nerve endings in the heart That would induce platelet aggregation locally around that ischemic area. I think that platelet aggregation is secondary to severe ischemia. If we start to reduce heart work and thus the metabolic demand by e g beta-blockade and try to keep coronary flow as good as possible that would be the first thing to do Then maybe aspirine could be added to prevent platelet aggregation.

Dr Poupa

I would like to add to Dr Hjalmarson s presentation and to the comment of Dr Morgan some few points from the comparative point of view Some animals are well equipped for survival in anoxic conditions. When they are subjected to both acute (divers) or chronic (hibernators estivators) anoxic episode they reduce cardiac work. Bradycardia seems to be the main natural defense mechanism which they are using to preserve cardiac integrity under anoxia. The second factor of importance is the simultaneous body cooling How their tissues – including the heart muscle – cope with the excess of H is far to be clear

Dr Braunwald

Dr Ross in La Jolla has measured myocardial oxygen consumption in patients with heart failure using techniques that are probably better than the ones that have previously been employed. He found that when correcting for a wall tension and correcting these measurements for 100 grams of muscle there was a reduced oxygen consumption which correlated with the reduction of the calculated velocity of circumferential fibers in the more slowly contracting failing heart muscle A variety of studies have demonstrated that there is a reduction of some contractile ATP ase in heart failure – whether it is myosin ATP:ase myofibrillar or actomyos n ATP ase and Dr Arnold Katz has suggested that the reduction of the velocity of reaction in heart failure might actually serve as a very im-

portant protective device because it would prevent myocardial oxygen consumption from arising and thereby prevent ischemia from advancing. That is as close a formulation that I have heard to yours but I do not think that it is formulated in precisely those terms.

Dr Kjekshus

A small comment to Dr Hjalmarson. I have been interested in the same issue because release of free fatty acids is provoked by the presence or the release of norepinephrine inside ischemic area. By inhibiting lipolytic activity the ischemic injury is reduced following coronary occlusion using the ST-segment mapping. However inhibiting lipolytic activity in reserpinized dogs did not modify the ST-segment elevation following coronary occlusion. I think this is related to the absence of norepinephrine in this area

Dr Mjos

Just one single question. What is known about the effect of long-term beta-receptor blockade on plasma concentrations of triglycerides or cholesterol Are there any hard data on long-term treatment

Dr Werko

It depends on what you mean with long-term treatment.

Dr Mjo

Let us say 1 years then

Dr Werkö

In the study in Goteborg when we did the follow-up of the postmyocardial infarction these values were followed There was no difference between placebo and beta-blockade We did also other metabolic studies like glucose tolerance and so on and nothing happened at all

Dr Gudbjarnason

I have some information that might be of interest here We have been studying the problem of tissue repair following infarction and we have been attempting to modify the infarct size. One of the problems that we ran into was that our reference tissue the non-infarcted myocardium, was also affected by the experimental procedure.

In a study where the infarct was made relatively large at least 20 per cent of the total left ventricle we measured several biochemical parameters for days and weeks after coronary occlusion. We observed biochemical changes in the non-infarcted myocardium that seem to be of considerable importance.

The high-energy levels (ATP and CP) of non-ischemic surrounding myocardium diminished significantly 24 hours after coronary occlusion and returned slowly to normal levels in 4 weeks (Gudbjarnason *et al* J Mol Cell Cardiol (1971) 2, 253-276) The norepinephrine level of non-infarcted muscle diminished in 10 days and returned to normal in 6 weeks

It is important to turn our attention to the non-infarcted, surviving and working muscle Chemotherapy of myocardial infarction should also be aimed at support for the surviving non-infarcted muscle as well as reduction of infarct size In a study attempting to influence infarct size by treatment with anabolic agents we observed a significant reduction in scar formation. Measurements of hydroxyproline and collagen content of infarcted tissue illustrated a 25 per cent diminution in infarct size and scar formation in dogs treated with anabolic steroids (Gudbjarnason *et al* Recent Advances in Studies on Cardiac Structure and Metabolism, Vol 1 1972, p 439-446) The anabolic agents may also serve to stimulate the development of compensatory hypertrophy in non-infarcted myocardium and may aid in restoration of myocardial function.

Dr Braunwald

Two interesting points. I think there have been a number of studies now that have shown that the functional changes in the normal portion of heart with regional ischemia occur very quickly and that they are fairly profound. Particularly with large infarcts there is considerable increase in the diastolic fiber length and increase in contractility of the normal tissue so there is a lot going on in the remainder of the heart which has been largely neglected and I think that this is an important point. About a year ago a number of us, Dr Morgan Dr Sobel and I were involved in a symposium with Soviet scientists and they directed a great deal of attention to the histochemistry of this portion of the heart and they reported profound histochemical changes.

Dr Werkö

Well ladies and gentlemen it is not a long time since an infarct was an infarct – that is dead tissue – and that was that. This meeting has demonstrated the importance that we as clinicians change from being dependent on the morbid anatomists and firstly I think rely more on clinical reality as observed, and secondly move towards biochemistry and physiology in our interpretation of clinical events. This is the only way of improving both the diagnostic procedures, our therapy and also ways of determining the prognosis

I went to this meeting with great expectations because we had succeeded in getting so many high quality scientists coming to take part. We are all thanking those who have contributed to these two days sitting here inside when the sun is shining outside and some of you on the way home from a long journey to Russia. We thank you all for the work you have done to try to explain what is really going wrong in ischemia and anoxia. Partly you have also increased the confusion in our minds. I think somebody said he was more "foggy" now than before and to really know what is dead tissue and what is living tissue has not become easier

I do not believe that any important questions have been sol ed The importance of the meeting has been that the questions have been asked and I think we ha e heard many provocative statements. We have now a chance of going home and contemplate what we have heard or continue with the work to answer at least some of the questions put forward.

I hope that the discussions have stimulated not only us Scandinavian clinicians but also our distinguished guests and may have given some new ideas worth testing in the experimental model To use Dr Braunwald's description earlier today of scientific work, I hope that you have been on an interesting voyage for some on an altitude of 35 000 feet, others more close to the ground, but I hope that all of you have found the ground covered interesting enough to come back to.

With this I close the meeting, but before that I also want to thank ICI for sponsoring the meeting and for arranging it in such a nice way in the beautiful city of Copenhagen and this nice place. Thank you very much.

Acta Medica Scandinavica

SUPPLEMENTUM 588

The Oslo Study

Cardiovascular disease in middle-aged and young Oslo men

By P. Leren, E. M. Askevold, O. P. Foss, A. Frølli,
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The Oslo Study

Cardiovascular disease in middle-aged and young Oslo men.

by

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OSLO 1975

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Contents

I Introduction

Since World War II there has been an alarming increase in the development of coronary heart disease (CHD) in Norway In Oslo cardiovascular diseases are the main cause of death, and was responsible for 52,6 per cent of all cases in 1970 about half being due to CHD At ages below 65 years the male incidence of coronary deaths is four times that of females. Elevated levels of blood cholesterol hypertension and the habit of cigarette smoking are believed to represent the main risk factors of CHD in Oslo However variability in extent and severity of atherosclerosis among individuals subjected to a uniform environment long has been a prominent feature of atherosclerosis. Varying levels of susceptibility tend to blur association between environmental agents and disease Elucidation of cause – and effect mechanisms and development of means to control them may provide specific tools which may be applied to healthy high-risk individuals. These high-risk individuals can be reached in different ways – by family physicians, by medical examination in school military service and at the working place The best way to obtain medical contact with the great majority of high-risk individuals, will be through mass screening of selected groups.

However whether identification and control of identified CHD risk factors in healthy individuals will result in reduced future incidence of CHD has not unequivocally been shown. Thus there is still a need for controlled intervention studies.

II Design of the study

The Oslo Study is a combined epidemiological – preventive investigation of CHD and other atherosclerotic diseases in young and middle-aged men in Oslo. The study is the joint effort of very many persons, laboratories and institutions. It is directed by a steering committee appointed by the Alderman for Hospitals in Oslo. A detailed protocol for the study has been worked out.

A METHODS

1 Screening procedures

All Oslo men aged 40-49 years and a 7 per cent random sample of men aged 20-39 were mailed a questionnaire and offered an examination allowing the determination of the following variables: known presence of CHD, cerebral vascular disease (CVD), hypertension, atherosclerous obliterans of the lower limbs (ASO), diabetes or symptoms of CHD and ASO. Tobacco smoking, physical activity at work and at leisure, subjective feeling of "stress" were also registered.

The order of examination was men born on the first in a given month, then on the second, the third etc. In the age group 20-39 the men born on the third and on the eighteenth of each month received an offer for examination. The men were asked to meet within one week after reception of the invitation. Those who failed to meet were not recalled.

The one-page questionnaire was made as simple as possible. It was printed in two colours and allowed only yes- and no- answers. The answers were directly punchable.

The WHO manual for epidemiological studies (3) was used ... mod... ...alth ...ugh modified in some respect.

The first ... ...nation included ... ... of questi...nna... ...ot ... ...g ... bl...od sample for total cholesterol, triglycerides and glucose, measuring of height, weight, blood pressure sitting after 5 minutes rest, and a miniature chest x-ray. These examinations were performed by nurses, who all had been thoroughly trained for the task.

At regular intervals their technique of blood pressure measurement was controlled by using tapes with Korotkoff sounds. The first and the fifth phase were used to determine systolic and diastolic blood pressure, respectively. The other procedures were also constantly supervised.

The examination started May 1972 and the screening phase was finished December 1973. By that time 17965 men had been examined. All re-examinations and follow-up studies according to the protocol have been carried out in Ullevaal Hospital, mainly in the Medical Out Patient Clinic, except for the hypertensive men, age 20-39, who for special reasons have been followed up in Aker Hospital, Oslo.

Chemical laboratory methods

Those invited to the screening examination were not asked to be fasting. The samples were collected between 8 a.m. and 3 p.m.

The blood was collected from an antecubital vein using a tourniquet while the subject was sitting. The blood was collected in Vacutainer[TM] 10 ml tubes containing 5 mg of sodium iodoacetate as preservative for glucose. The determinations were done in the blood serum.

The chemical determinations were done at the Central Laboratory, Ullevaal Hospital, Oslo. The concentration of total cholesterol and triglycerides was estimated simultaneously by a Technicon AutoAnalyzer[TM] according to Technicon work sheet AA II ...3 and AA II ...4 May 1971. The procedure was modified slightly in order to counteract the negative

interference which the water in the sample of serum causes in the determination of cholesterol.

The cholesterol values obtained with this method are as an average 8 mg/100 ml (0,_ mmol/l) lower than obtained with the method of Carr and Drekter (6). However they are about 20 mg/100 ml (0,5 mmol/l) higher than obtained with a gaschromatographic method introduced in 1973 (4) and the enzymatic method (14) introduced in 1974 The triglycerides values obtained with this method are as an average 0,2 mmol higher than obtained with an enzymatic method (Biochemica test combination Cat. no. 15989 Boehringer Mannheim G.m.b.H.). The concentration of glucose was estimated by Technicon AutoAnalyzer II™ according to Technicon work sheet AA II – o_ – Nov 1970 The determination is based upon the reducing properties of glucose and yields about 18 mg/100 ml (1.0 mmol/l) higher values than the glucose oxidase method.

The stability of the analytical procedures was controlled continuously by inserting reference sera among the actual sera (at place 20 – 40 – 60 – and so on). The determination of cholesterol and triglycerides was controlled by using the same lot number of Technicon Reference Serum during the entire screening period The determination of glucose was controlled by Seronorm™ Nyco Oslo The mean of the results obtained with the reference sera were every day compared to stated values based upon the mean of the results obtained during a period of two months prior to the screening period. We accepted a difference between the mean of the day and the stated value until 8 mg cholesterol/100ml, 0.08 millimol triglycerides/l and 4 mg glucose/100 ml. When the differences were ±9 – 1? mg cholesterol/100 ml 0.09 – 0 1 mmol triglycerides/l and – 5 – 6 mg glucose/100 ml corrections were made for all actual samples. In case of larger differences the samples were re analyzed.

The results obtained at Ullevaal Hospital were compared to those obtained at Lipid Research Clinic, George Washington University Washington D C By generous courtesy of this institution frozen samples of human plasma were sent to Ullevaal Hospital. The cholesterol values found in Oslo were as an average 15 mg/100 ml (0 4 mmol/l) higher than those found in Washington D C The chemical method and analytical instrument are the same at both places. However the way of standardization differs. (11).

In the Lipid Research Clinic Program the standardization is chosen so the cholesterol values agree with those obtained with the method of Abell et al. (1)

When the calculation is done in the same way both places, no significant difference exists.

In most of the methods used the world over including the method used in Lipid Research Clinic Program and the Oslo Study esterified cholesterol yields somewhat higher values than free cholesterol. It was not common to correct for this difference when the Oslo Study started in 1972 However such correction was introduced in Lipid Research Clinic Program. Therefore the cholesterol values reported from this project, are about 15 mg/100 ml (0 4 mmol/l) lower than reported from the Oslo Study The Oslo Study participates in The Cooperative Cholesterol Standardization Program which is organized by WHO Regional Lipid Reference Center in Prague It has been demonstrated that values found in Oslo as an average are 11mg/100 ml (0,3 mmol/l) higher than the expected values which are based upon the method of Abell et al. (1).

The triglycerides values found in Oslo as an average are 0 1 mmol/l higher than those found at Lipid Research Clinic Washington D C It is assumed that the difference depends upon difficulties in the analytical procedure We have never been satisfied with the method for determination of triglycerides. Great efforts have been spent but the reproducibility and stability have *never reached* the desired level.

The details of the methods used in the Oslo Study their advantages and disadvantages are given in a separate protocol.

3 EDP-procedures.

A protocol was set up giving detailed instructions for the reception of questionnaires and control of EDP procedures. A daily self check by the assistants, followed by a weekly control by a physician was arranged. All ques-

tionnaires were each fortnight in a number of 500 – 1000 sent to the EDP – Division for double punching. A manual check of each questionnaire against control lists was performed. Each questionnaire was controlled for logical inconsistencies, and the corrected questionnaires were repunched. All data of one person was linked to his person number of 11 figures, including 6 birthdate figures. The cards with examination data including results of blood samples were coupled to the questionnaire results. A computerized control program for remaining inconsistencies of questionnaire answers, faulty person numbers, extreme values for blood analysis, blood pressure and risk score was carried out leading to correction lists at regular intervals. These lists were compared with original questionnaires and basis cards both for clear text and punch holes.

Lastly the final controlled and corrected tables were processed.

4 Risk score calculation

The risk score table includes cholesterol, systolic blood pressure and number of cigarettes. The risk score table for cholesterol and blood pressure has been constructed according to the results of the prospective study of Westlund and Nicolaysen (15). In this study 3751 Oslo men aged 40-49 were followed for 10 years. The risk of smoking cigarettes has been analyzed in a prospective study in Oslo in 17000 men (12).

Age, although an important risk variable, was deliberately omitted from the score computations. According to results of these studies, a multiplicative risk score table has been constructed. This risk model is based on mutual independance of the actual risk variables. Cholesterol < 190 mg per 100 ml was given the score of 1.0 gradually rising to 5 at a cholesterol value of ≥ 450. Systolic blood pressure < 135 mm Hg got the score of 1.0 rising to 4.5 at a blood pressure ≥ 170.

Finally non-cigarette smokers got the score 1.0 with a gradual increase to 4.0 for smokers of ≥ 5 cigarettes per day. Thus the lowest and the highest obtainable risk score is 1 and 450 respectively. A couple of examples will illustrate how the risk score table work. Cholesterol of 50 and systolic blood pressure of 150 in a man smoking 10 cigarettes a day will give a risk score of 13.5, while 350, 160 and 20 for cholesterol, systolic blood pressure and cigarettes, respectively, give a score of 144.0.

5 Statistical methods

Differences between means have been tested by a modified Student's t test accounting for unequal variances and numbers of groups. P values below 0.01 were regarded statistically significant.

All correlation coefficients are based on individual observations. The significance of the coefficients were tested by the Student's t test.

B CONSEQUENCES OF SCREENING

Based on the results of the screening examinations the men were taken care of in the following ways. Positive findings on X-ray led to re-examination at the Tuberculosis Division according to usual procedures.

Men with a blood glucose ≥ 170 mg per 100 ml were recalled for further examination and treatment as necessary.

Men born during 1933 5 with a systolic blood pressure ≥ 150 mm Hg were recalled for further examination and treatment as necessary.

Men born during 1933 4 with serum cholesterol ≥ 350 and men born during 1943 5 with serum cholesterol ≥ 325 mg per 100 ml were recalled for further examinations and treatment as necessary.

Men with questionnaire answers suggesting angina pectoris or intermittent claudication were recalled for further examination and treatment as necessary.

Men born during 19_3 – 32 with systolic blood pressure ≥ 150 mm Hg who did not satisfy the criteria of the preceding categories, were recalled for the first re-examination at Ullevaal Hospital.

Men born during 19_3 3 with a risk score ≥ 10 or with cholesterol ≥ 380 or systolic blood pressure ≥ 160 were recalled for the first re-examination.

Men born during 19 3 3 who were non-smokers and never-cigarette smokers and who had a serum cholesterol < ? 8 mg and a blood pressure < 141/94 were recalled for the first re-examination.

The recall letters were sent out not more

than 3 weeks after the screening examination Details for the first and the second re-examination will be given in subsequent reports.

Figure 1 is a flow chart demonstrating the order of examinations and the establishment of different patient – and study groups.

C ON-GOING STUDIES

a) The effect of anti-hypertensive drugs in men aged 40-49 with mild hypertension will be studied in a controlled clinical trial

b) In another controlled clinical trial it will be studied Whether it is possible to get symptom-free men, aged 40-49 who have high cholesterol and / or cigarette consumption, to change these risk factors by means of dietary and hygienic advice if an effect is obtained, whether it is possible to maintain it for at least 5 years, if this effect can be maintained whether the incidence of clinical disease is affected.

c) By means of exercise electrocardiography of symptomfree men with high and low risk variables, and of men with recognized CHD it will be studied how the findings relate to risk variables how the findings relate to future incidence of CHD of the symptom-free men how the findings are influenced by the treatment given in the controlled trials mentioned above

d) A special psychological and physiotherapeutical examination of a sample of men with various risk variables will be performed.

D FUTURE STUDIES

a) Further analysis of the screening data, specially the interrelationship of three and more variables. (By means of multiple regression analysis)

b) Study of the clinical data at screening and follow-up examinations for the purpose of determining the prevalence of men who satisfy various criteria for treatment

c) Linkage of the screening data to the individual data in the 1970 census such as education, type of work, income housing etc. both for attendants and non-attendants (test for possible selection at screening).

d) A cause-specific mortality follow-up will be achieved by means of the person number

A CHD morbidity follow-up is planned by means of a myocardial infarction register

e) Quantitative study of atherosclerotic lesions in fatal cases among the men invited for the screening (in collaboration with the International Atherosclerotic Center in New Orleans).

III Results of screening

Table 1 presents the number of men met at screening and the percentages of attendance in 5-year age groups. The attendance is best in the age groups 40-44 and 45-49 and much lower in the younger age groups.

Table 2 shows the prevalence of some diseases previously diagnosed according to information given by the men. The criteria of the diagnoses are unknown. In this table the same man may have been listed under more than one diagnosis. Hypertension and CHD are most frequent.

A Living habits

In tables 3 and 4 data for the degree of physical activity at work and at leisure respectively are given. As much as half the men have sedentary work and only 3 per cent have really heavy work. At leisure however only 0 per cent are sedentary while as much as about 80 per cent enjoy some physical activity. Age difference with regard to physical activity is not marked.

Table 5 lists the answers on some questions about subjective feeling of mental tension and strain. The table reveals a trend towards increasing mental stress with increasing age.

Tables 6 and 7 present smoking habits. The number of daily smokers (table 6) increases with age. More than 90 per cent of the daily smokers are inhaling, and 91 per cent of the daily smokers in the age group 70-79 are cigarette smokers compared to 8 in the age group 40-49. Pure pipe or cigar smoking is rare only 1 6 per cent in the young and 3 8 per cent in the age group 40-49.

As much as 36 1 per cent in the age group 70-79 have never smoked cigarettes. In the age group 40-49 this figure is only 16.9 per cent. In the age groups 70-79 and 40-49 1 9 and 4,6 per cent respectively had quit smoking cigarettes. Only about one fourth of cigarette smokers are using filter cigarettes.

Table 7 presents the amount of tobacco consumption in pipe smokers and the number of cigar smokers. As seen in tables 6 and 7 most pipe smokers are also smoking cigarettes, while cigar smokers are rare.

B Main group

Table 8 presents the results of testing the homogeneity of the men who met at screening. Here the levels of some risk variables in patients who previously had certain diseases diagnosed are compared with the risk factor levels of the men without symptoms or known diseases. A systematic and significant difference is revealed showing higher risk variables in the patients as compared with the healthy men. This last group is called the main group. Further tests revealed that those questionnaire-positive men with regard to possible angina and claudication in whom clinical examination failed to verify such ailments did not differ from those in the main group with regard to the risk variables studied. These men have been included in the main group for further analysis. This group is believed to represent the normal healthy men of whom however many exhibited high CHD risk factors of which they were ignorant. This subgroup of high risk persons may probably profit by active intervention in order to change their risk profile.

Table 9 shows that the youngest are 3 7 cm higher and 4 1 kg lighter than the oldest.

Table 10 presents the age gradient for six variables. There is a gradual increase in these variables by age the most prominent feature being the cholesterol increase from 199 mg per 100 ml at age 0- 4 compared with 70 at age 45-49. The triglycerides, however did not increase after the age of 35. It should be remembered that the triglyceride values are non fasting.

The age dependance of cholesterol and blood pressure is graphically shown in Fig. 2 The age gradient for cholesterol is more pronounced than for blood pressure

In cross tables 11 18 the degree of physical activity at work and at leisure is compared to the level of serum cholesterol systolic and diastolic blood pressure and of risk score

The tables are so constructed as to make it possible to compare different degrees of activity at work and at leisure simultaneously with the actual risk factor

Tables 11 and 12 present the cholesterol data. In both age groups serum cholesterol increases with increasing physical activity at work, while the opposite holds for leisure activity

In tables 13 16 the blood pressure data are presented. Both systolic and diastolic pressures tend to be increased with high work activity while leisure activity is associated with lower blood pressure

Tables 17 18 compare physical activity with the risk score value Again, increasing leisure activity is associated with reduced CHD risk, while increasing activity at work is associated with increased risk score value

Thus, tables 11 18 reveal that the most preferable levels of serum cholesterol, blood pressure and risk score seem to be found in those with sedentary occupation while they expose great physical activity during leisure time.

Tests revealed no significant correlation between the feeling of mental stress and CHD risk variables (data not given).

In tables 19-21 six variables are related to number of cigarettes in present cigarette smokers and compared to the values of men who have never smoked cigarettes. All variables tend to show increasing values with increasing number of cigarettes, while the values are low in those who had never smoked cigarettes and compare well with those only smoking 1-4 cigarettes per day

In cigar and pipe smokers (table 22) however such an increase in the levels of these variables with increasing tobacco consumption is not found, nor is there any significant difference between pipe smokers and cigar smokers with regard to these variables.

Table 23 elucidates the influence on serum cholesterol of duration of cigarette smoking.

It is shown that long-term cigarette smokers have higher cholesterol values than short term smokers.

Thus, it is shown that the highest serum cholesterol values are found in the long-term heavy cigarette smokers.

Table 24 presents some examples of seasonal variation of some variables. Serum cholesterol, and consequently also risk score show significant deviations. Also systolic blood pressure and blood glucose show significant deviations, though not so marked as for serum cholesterol. In figure 3 the seasonal variations of serum cholesterol during 19 months are graphically presented. The lowest values are found in May June and the highest in November December January

In table 25 seasonal variations in physical activity and in the feeling of stress are presented. There is no consistent seasonal variation neither in work activity nor leisure activity Nor is there any marked tendency to seasonal variation in the subjective feeling of mental stress

Intercorrelations of risk variables.

Table 26 presents an overall survey of correlation coefficients and their t values for significance between pairs of risk variables.

In figures 4-16 some of these correlations are graphically shown. Unless otherwise stated, these correlations are statistically significant.

Figures 4-8 correlate serum cholesterol and the variables of body weight, relative body weight, duration of cigarette smoking, blood pressure and blood glucose

In figures 9-16 triglycerides, blood pressure blood glucose and risk score is correlated to absolute and relative body weight.

TABLE 1 Number of men met at screening by age.

Age		attendance (per cent)
20–24	382	33,1
20–29	591	43,3
30–34	393	48,9
35–39	397	61,6
40–44	7427	65,0
45–49	8775	64,9
Total	17965	62,1

TABLE 2 Distribution of previous diagnoses according to information given by the men

Age		Myocard infarct	Angina pectoris	Other heart d.	Atheroscl of legs	Stroke	Diabetes	Hypertension
20–29	n	0	0	8	0	0	3	5
30–39	n	1	4	7	2	0	4	6
40–49	n	164	194	191	41	36	153	446
	%	1,0	1.1	1.1	0.[illegible]	0.[illegible]	0.9	2.?
Total	n	165	198	[illegible]06	43	36	160	45?
	%	0.9	1.1	1.1	0.[illegible]	0.[illegible]	0.8	[illegible]

TABLE 3 Physical activity at work

Age		Sedentary	Moderate activity	Intermediate activity	Great activity	Unanswered	Total
20–29	n	519	261	174	18	1	973
	%	53,3	26,8	17,8	1,8	0.1	100.0
30–39	n	397	235	133	21	4	790
	%	50.[illegible]	29.7	16,8	2.6	0,5	100.0
40–49	n	7894	4757	2792	609	150	16202
	%	48.7	29,3	17,2	3.7	0.9	100,0
Total	n	8810	5253	3099	648	155	17965
	%	49,0	29,2	17,2	3,6	0,8	100.0

Definitions

Sedentary: Mostly sedentary work as writing, table work, [illegible] maker work, mounting
Moderate activity: Work which demands much walking as shopman, light industry work, teaching
Intermediate activity: *Work which demands much walking and lifting as postman, heavy industry* work, building work
Great activity: Heavy manual labour as lumber, farmer, heavy building work

TABLE 4 Physical activity at leisure

Age		Sedentary	Moderate activity	Intermediate activity	Great activity	Unanswered	Total
2[illegible]		173	501	219	80	0	973
		17.7	51,4	22,5	8,2	0,0	100.0
30–39		169	433	163	25	0	790
		[illegible]1,3	[illegible]4,8	[illegible]0,6	3.1	0.0	100.0
40–49	n	[illegible]446	9631	2846	[illegible]56	23	16 [illegible]02
	%	1[illegible]	59.4	17.5	1,5	0.1	100.0
Total	n	[illegible]	10 [illegible]65	3228	361	23	17965
	%	[illegible]	[illegible]	17,9	2,0	0.1	100.0

Definitions

Sedentary: Reading [illegible] television [illegible] other sedentary [illegible] occupation
Moderate activity: Walking, bicycling [illegible] other forms of physical activity including walking or bicycling to and from [illegible] workplace [illegible] walk for [illegible] least 4 hours [illegible] week
Intermediate activity: [illegible] 4 hours [illegible] week
Great activity: [illegible] sports regularly several times [illegible] week

TABLE 5 Subjective feeling of stress

Age		Psychic tension	High strain	Press on deadlines	Contented, peaceable
20–29	n	179	471	371	276
	%	18 4	48,5	38,2	29,4
30–39		194	436	389	210
	%	24,6	55,6	49 7	28,0
40–49		4533	9065	8227	4425
	%	28,0	56,2	51,1	27,8
Total		4906	9972	8987	4911
	%	27,3	55,8	50 4	27,9

Definitions:

Psychic tension. Have you during the last year had a feeling of increased psychic tension or irritation?
High strain. Do you regard yourself to be a person who stresses yourself and often chooses high speed?
Press on deadlines. Do you think it is more than usual pressure on deadlines in your working situation?
Contented, peaceable. Or do you regard yourself as a person who gives up high speed and personal ambitions in order to enjoy peaceful and quiet life?

TABLE 6 Smoking habits by age.

Age		Daily smokers	Inhalers	Daily cigarette smokers	Daily cigarette smokers (filter)	Daily pipe-cigar smokers (only)	Ex-cigarette smokers	Never smoked cigarettes
		% all men	% daily smokers	% daily smokers	% daily cigarette smokers	% all men	% all men	% all men
20–29		493	464	450	119	16	126	354
	%	50,6	94,3	91,2	25,8	1,6	12,9	36,3
30–39		404	365	364	91	16	172	214
	%	51,1	90 7	90,0	24,8	2,0	21 7	27,0
40–49		9135	8346	7509	1774	621	3992	3075
	%	56,3	91,8	82,2	23 4	3,8	24,6	18,9
Total	n	10032	9175	8323	1984	653	4290	3643
	%	55,8	91,9	82,9	23,6	3,6	23,8	20,2

TABLE 7 Pure pipe and cigar and combined pipe and cigar smokers by degree of weekly tobacco consumption.

AGE	Tobacco consumption in pipe smokers. < 25 g		25–100 g		≥100 g		Cigar smokers	
	Pure	Comb.	Pure	Comb.	Pure	Comb.	Pure	Combined
20 29	9	56	23	41	0	1	6	15
30 39	6	30	19	45	1	3	8	20
40–49	124	677	1031	1773	106	182	251	481
Total	139	763	1073	1859	107	186	265	516

TABLE 8. Test of homogeneity Risk variables in various disease groups and the main group

		Known myocard. infarct and angina pect.	Other heart diseases	ASO	Stroke	Diabetes	Hypertension	Use of Nitrogl.	Angina and/or claudicat. pos. questionnaire	Main group
Cholesterol mg/dl	n	307	191	41	36	153	446	154	822	14816
	MV	291.2	260.4	286.0	280.5	274 8	281 9	296.3	273 6	267.2
	SD	58.8	44 7	42 0	57 4	51 1	48 6	57 4	52 4	48.5
Triglycerides mM/l	n	307	191	41	36	153	446	154	822	14815
	MV	2 77	2.31	2.64	2.29	2.57	2.81	2 96	2.53	2.2
	SD	1 47	1 97	1.27	1 11	2.36	1.82	1.55	1.68	1.37
Glucose mg/dl	n	307	191	41	36	153	446	154	822	14813
	MV	106.5	102 7	101 0	111 1	191.8	109.6	107.4	104 1	10..3
	SD	29.9	18 1	14 4	25 4	93 9	29 0	34.2	25 4	18.5
Systolic BP mm Hg	n	307	191	41	36	153	446	154	822	14815
	MV	140 4	137 7	138.5	144 7	143 4	157.3	139 7	136 9	134 9
	SD	18.3	18 7	16.8	22 4	23.2	19 4	18 4	18.0	15 6
Diastolic BP mm Hg	n	306	190	41	36	153	446	153	822	14815
	MV	91 9	87 1	87 4	94.2	89 6	102.3	91.3	88.2	86.2
	SD	11 7	11.2	10 4	13.5	13.2	10 8	10 9	11.9	10 6
Risk score	n	307	191	41	36	153	446	154	822	14816
	MV	17 35	10 6	15 77	21.46	17 4	22.22	19.27	14 67	10 2
	SD	22 80	12.61	17 16	22.72	28.3	26 4	26.8	20.5	15.3

TABLE 9 Height and weight by age Main group

Age	n	Height	Weight
		(cm) (SD)	(kg) (SD)
29 24	376	180 9 (6.9)	73.5 (9.3)
5 29	374	179 3 (6 7)	75.3 (9 9)
30 34	378	178.5 (6 9)	76.2 (9 8)
35 39	39	178 7 (6 9)	77.8 (10.2)
40 44	6909	177 8 (6.3)	77 7 (10 1)
4 47	7706	177 (6.3)	77 6 (10 1)
Total	17 5	177 7 (6 3)	77 5 (10 1)

TABLE 10. Main group. Risk variables by age.

Age		Cholesterol mg/dl	Triglyc. mM/l	Glucose mg/dl	Systolic BP mm Hg	Diastolic BP mm Hg	Risk score
	n	376	376	376	376	376	376
20–24	MV	199 7	1.67	94.5	128.8	75 7	3.0
	SD	38.9	0.89	13.5	11.6	9 1	3.5
	n	574	574	574	574	573	574
25–29	MV	223.9	1.79	96.3	129.6	79.3	4.5
	SD	46.5	0.96	15.2	11.9	9 4	6.3
	n	378	378	378	378	377	378
30–34	MV	242.7	1.98	97.5	130.6	81.2	6 1
	SD	47.9	1.03	13.5	12.4	9.7	8.0
		382	382	382	382	382	382
35–39	MV	248.7	2.17	98.8	132.1	83.5	7 7
	SD	45.9	1.50	16.2	13.5	9.9	9.3
	n	6909	6908	6908	6909	6909	6909
40–44	MV	263.5	2.21	101.8	134.0	85.8	9.5
	SD	49.5	1 47	16.6	14.8	10.6	15.0
		7906	7906	7904	7905	7905	7906
45–49	MV	270.4	2.23	102.9	135.6	86.6	10.7
	SD	47.4	1.28	20.0	16.3	10.7	15.6
		16525	16524	16525	16524	16522	16525
Total	MV	263.2	2.19	101.8	134 4	85.6	9 7
	SD	49.9	1.35	18.2	15 4	10.7	14.8

TABLE 11 Physical activity at ork and at leisure in relation to serum cholesterol.
Age 40–44 Main group.
(for definitions see tables 3 and 4).

Physical activity Work / Leisure		Sedentary	Moderate activity	Intermediate activity	Great activity	Unansw	Total
		729	411	311	70	10	1531
Sedentary	MV	268.6	270.6	270.9	276.8	305 4	270.2
	SD	55.5	47 7	47 1	53.6	45.5	51 7
		1979	1201	660	102	22	3964
Moderate activity	MV	262.1	264.3	268.6	272.0	271.9	264.1
	SD	48.5	51.6	50.4	46.5	50.0	49.8
		673	356	190	48	0	1267
Intermediate activity	MV	252.6	256.6	260.6	272.5	0.0	255 7
	SD	44.8	43.7	46.7	49 4	0.0	45.2
		75	33	24	7	0	139
Great activity	MV	242.2	244.5	267.3	241.7	0.0	247 1
	SD	38.9	36.2	40.9	42.8	0 0	39.5
		1	2	1	1	3	8
Unansw	MV	285.0	193.0	231.0	301.0	226.3	235.2
	SD	0.0	33.9	0.0	0 0	76.8	58.0
		3457	2003	1186	228	35	6909
Total	MV	261.2	263.8	267.9	272.8	277.6	263.5
	SD	49.2	49.2	48.8	49.3	51.7	49.2

TABLE 12 Physical activity at work and at leisure in relation t serum cholesterol.
Age 45–49 Main group.
(for definitions see tables 3 and 4)

Physical activity Work / Leisure		Sedentary	Moderate activity	Intermediate activity	Great activity	Unanswered	Total
Sedentary	n	633	438	354	78	14	1547
	MV	272.5	276.3	276.3	276 4	30 .3	274 9
	SD	48.9	52.0	42.8	44 4	79 4	48.7
Moderate activity	n	2310	1494	791	175	43	4813
	MV	268.7	269 7	274 6	275 6	27 .6	270.3
	SD	46 9	46.5	49.5	46 9	40 4	47.2
Intermediate activity	n	756	375	214	73	6	1424
	MV	262.7	270 9	67.8	79.3	45.5	266 4
	SD	44 4	45 9	50 1	55.9	38.1	46.5
Great activity	n	63	31	17	1	0	11
	MV	265.2	257 7	270 0	239	0.00	63 6
	SD	38.5	43 4	54.5	0.0	0 00	4..9
Unanswered	n	2	0	0	2	6	10
	MV	247.0	0.0	0 0	206 0	264 0	49.0
	SD	59.3	0 0	0.0	22.6	59 4	54.5
Total	n	3794	2338	1376	329	69	7906
	MV	268 1	271 0	273 9	276 1	275.5	270.4
	SD	46.6	47 2	48.0	48.3	52.2	47.3

TABLE 13 Physical activity t work and t leisure in relation t systolic blood pressure
Age 40–44 Main group
(for definitions see tables 3 and 4)

Physical activity Work / Leisure		Sedentary	Moderate activity	Intermediate activity	Great activity	Unanswered	Total
Sedentary	n	7 9	411	311	70	10	1531
	MV	133 7	134.2	136.3	138.6	129.0	134 6
	SD	15 1	14 9	16 7	13.5	10 0	15.3
Moderate activity	n	1979	1201	660	102	2	3964
	MV	133.2	134 4	135 9	136 4	135 7	134 1
	SD	14 5	15.5	14.8	16.2	19.5	15.0
Intermediate activity	n	673	356	190	48	0	1 67
	MV	132 9	133 7	134.2	132.3	0 0	133.3
	SD	13 8	13 4	13 9	13.2	0 0	13 7
Great activity		75	33	24	7	0	139
	MV	128 9	135.5	132.4	132.0	0 0	131.2
	SD	13 3	13.2	12.6	7.2	0.0	13 1
Unanswered		1	2	1	1	3	8
	MV	134 0	125 0	126.0	120 0	1 3.3	125.0
	SD	0 0	1 4	0.0	0 0	4.6	4 7
Total		3457	2003	1186	.28	35	6909
	MV	133 2	134	135 7	136.0	132.7	134.0
	SD	14 5	15 0	15 1	14.6	16 4	14.8

TABLES 14 Physical activity at work and at leisure in relation to systolic blood pressure.
Age 45–49 Main group.
(for definitions see tables 3 and 4).

Physical activity Work / Leisure		Sedentary	Moderate activity	Intermediate activity	Great activity	Unansw	Total
Sedentary	n	663	438	354	78	14	1547
	MV	135.4	135.6	137.9	139.6	133.1	136.2
	SD	16.0	15.3	16.0	16.3	15.0	15.8
Moderate activity		2309	1494	791	175	43	4812
	MV	134.7	135.9	138.1	137.6	132.1	135.7
	SD	16.4	16.5	17.3	17.6	17.8	16.7
Intermediate activity		756	375	214	73	6	1424
	MV	132.8	136.8	136.7	139.1	139.6	134.8
	SD	14.3	16.8	16.9	16.6	22.3	15.7
Great activity		63	31	17	1	0	112
	MV	136.5	133.6	135.0	150.0	0.0	135.6
	SD	17.8	12.2	14.6	0.0	0.0	15.9
Unansw		2	0	0	2	6	10
	MV	132.0	0.0	0.0	128.0	141.6	137.0
	SD	8.4	0.0	0.0	2.8	20.5	16.7
Total		3793	2338	1376	329	69	7905
	MV	134.5	136.0	137.8	138.4	133.8	135.6
	SD	16.0	16.3	16.9	17.0	18.0	16.3

TABLE 15 Physical activity at work and at leisure in relation to diastolic blood pressure.
Age 40–44 Main group
(for definitions see tables 3 and 4).

Physical activity Work / Leisure		Sedentary	Moderate activity	Intermediate activity	Great activity	Unansw	Total
Sedentary		729	411	311	70	10	1531
	MV	86.6	86.5	87.1	88.5	85.6	86.8
	SD	10.9	10.8	12.0	11.7	9.7	11.2
Moderate activity		1979	1201	660	102	22	3964
	MV	85.7	85.8	86.2	85.9	90.6	85.8
	SD	10.6	10.3	10.9	11.5	11.6	10.6
Intermediate activity		673	356	190	48	0	1267
	MV	84.8	84.4	84.9	85.4	0.0	84.7
	SD	9.8	9.2	11.0	8.0	0.0	9.8
Great activity		75	33	24	7	0	139
	MV	83.2	82.0	83.5	85.7	0.0	83.1
	SD	9.9	8.0	10.4	7.6	0.0	9.4
Unansw		1	2	1	1	3	8
	MV	86.0	73.0	86.0	86.0	82.0	81.2
	SD	0.0	1.4	0.0	0.0	7.2	6.6
Total	n	3457	2003	1186	228	35	6909
	MV	85.7	85.6	86.2	86.6	88.4	85.8
	SD	10.5	10.2	11.2	10.8	10.8	10.6

TABLE 16 Physical activity at work and at leisure in relation to diastolic blood pressure.
Age 45–49 Main group
(for definitions see tables 3 and 4).

Physical activity Work / Leisure		Sedentary	Moderate activity	Intermediate activity	Great activity	Unansw	Total
Sedentary	n	663	438	354	78	14	1547
	MV	87.7	87.6	88.0	88.0	89.8	87.8
	SD	11.1	10.8	11.0	9.9	12.0	10.9
Moderate activity	n	2309	1494	791	175	43	4812
	MV	86.3	86.8	87.2	86.6	86.1	86.6
	SD	10.5	10.9	10.8	11.7	11.8	10.8
Intermediate activity	n	756	375	214	73	6	1424
	MV	84.8	86.1	86.8	87.8	90.0	85.6
	SD	9.3	10.4	10.9	11.0	16.7	10.0
Great activity	n	63	31	17	1	0	112
	MV	85.8	84.7	82.1	78.0	0.0	84.8
	SD	11.0	8.2	8.8	0.0	0.0	9.9
Unansw	n	2	0	0	2	6	10
	MV	81.0	0.0	0.0	83.0	90.0	86.8
	SD	7.0	0.0	0.0	4.2	14.0	11.6
Total	n	3793	2338	1376	329	69	7905
	MV	86.2	86.8	87.3	87.2	87.5	86.6
	SD	10.4	10.8	10.8	11.1	12.3	10.7

TABLE 17 Physical activity at work and at leisure in relation to risk score
Age 40–44 Main group
(for definitions see tables 3 and 4).

Physical activity Work / Leisure		Sedentary	Moderate activity	Intermediate activity	Great activity	Unansw	Total
Sedentary	n	729	411	311	70	10	1531
	MV	11.6	11.6	13.5	14.6	16.7	12.1
	SD	20.8	14.8	19.4	14.6	14.6	18.7
Moderate activity	n	1979	1201	660	102	22	3964
	MV	8.6	9.7	11.2	12.2	13.1	9.5
	SD	12.6	14.7	18.1	13.9	16.6	14.4
Intermediate activity	n	673	356	190	48	0	1267
	MV	6.5	7.6	7.7	8.1	0.0	7.0
	SD	10.8	13.7	8.7	7.0	0.0	11.3
Great activity		75	33	24	7	0	139
	MV	4.0	4.4	6.2	2.9	0.0	4.4
	SD	3.8	3.6	4.6	1.7	0.0	3.9
Unansw	n	1	2	1	1	3	8
	MV	10.9	2.8	2.0	9.8	4.0	5.0
	SD	0.0	1.7	0.0	0.0	4.0	4.0
Total	n	3457	2003	1186	228	35	6909
	MV	8	9.6	11.1	11.8	13.3	9.5
	SD	14.3	14	17.1	12.7	15.3	14.9

TABLE 18. Physical activity at work and at leisure in relation to risk score.
Age 45–49 Main group.
(for definitions see tables 3 and 4).

Physical activity Work / Leisure		Sedentary	Moderate activity	Intermediate activity	Great activity	Unansw	Total
Sedentary	n	663	438	354	78	14	1547
	MV	13.4	12.4	13.9	15.1	21.2	13.4
	SD	22.2	19.0	17.2	16.8	31.6	20.1
Moderate activity		2310	1494	791	175	43	4813
	MV	9.4	10.6	12.6	14.5	12.1	10.5
	SD	12.7	13.5	17.5	28.2	13.7	14.7
Intermediate activity		756	375	214	73	6	1424
	MV	7.2	10.5	10.8	11.6	10.7	8.9
	SD	8.9	14.9	13.9	16.0	12.5	12.1
Great activity	n	63	31	17	1	0	112
	MV	8.4	6.4	8.6	5.8	0.0	7.8
	SD	18.3	8.0	13.0	0.0	0.0	15.1
Unansw	n	2	0	0	2	6	10
	MV	5.2	0.0	0.0	1.5	12.6	8.9
	SD	5.1	0.0	0.0	0.5	12.7	10.8
Total	n	3794	2338	1376	329	69	7906
	MV	9.6	10.9	12.6	13.9	13.9	10.7
	SD	14.4	14.9	16.8	23.4	18.6	15.5

TABLES 19 Risk variables in men who never smoked cigarettes, and in present cigarette smokers by number of cigarettes.
Age 40–49 Main group

Number of cigarettes per day

		1–4	5–9	10–14	15–19	20–24	25	Unansw	Total	Never cigarette smokers
Cholesterol mg/dl	n	503	1265	2176	1380	1014	436	8	6782	2881
	MV	259,0	269,7	274,3	271,9	275,7	272,8	281,5	271,9	259,8
	SD	47,7	51,5	49,8	47,7	49,6	47,0	57,3	49,5	46,7
Triglycerides mM/l	n	503	1265	2176	1380	1014	436	8	6782	2880
	MV	2,1	2,2	2,4	2,4	2,5	2,5	3,2	2,4	2,1
	SD	1,1	1,1	1,4	1,2	1,6	1,5	1,3	1,3	1,5
Glucose mg/dl		503	1265	2175	1380	1014	436	8	6781	2880
	MV	101,1	100,8	102,3	103,0	103,7	106,4	108,1	102,6	101,3
	SD	18,6	17,5	19,0	17,3	21,2	25,0	24,4	19,2	17,5
Systolic BP mm Hg		503	1265	2176	1379	1014	436	8	6781	2881
	MV	133,8	134,2	134,7	135,1	135,8	137,1	139,5	134,9	134,5
	SD	16,4	15,8	16,3	15,6	16,2	16,4	11,5	16,1	15,1
Diastolic BP mm Hg		503	1265	2176	1379	1014	436	8	6781	2881
	MV	85,0	85,4	85,5	85,6	86,8	87,8	87,0	85,8	86,9
	SD	10,8	10,6	10,9	10,6	10,9	10,3	6,6	10,8	10,3
Risk score		503	1265	2176	1380	1014	436	8	6782	2881
	MV	5,9	9,1	13,7	17,2	23,4	28,0	8,7	15,4	5,5
	SD	7,1	10,7	16,5	20,1	27,7	31,6	9,1	20,2	6,5

TABLE 20 Risk variables in men who never smoked cigarettes, and in cigarette smokers by number of cigarettes. Age 40–44. Main group

Number of cigarettes per day

		1–4	5–9	10–14	15–19	20–24	25	Unanw.	Total	Never cigarette smokers
Cholesterol mg/dl	n	204	523	985	677	506	213	5	3113	1504
	MV	254.6	269.1	270.0	269.2	273	268.8	296.8	269.1	254.9
	SD	51.3	58.0	51.6	47.5	49.2	47.3	70.4	51.4	47.2
Triglycerides mM/l	n	204	523	985	677	506	213	5	3113	1503
	MV	2.14	2.26	2.33	2.39	2.51	2.46	3.46	2.36	2.0
	SD	1.21	1.23	1.37	1.25	1.88	1.47	1.25	1.41	1.86
Glucose mg/dl	n	204	523	985	677	506	213	5	3113	1503
	MV	100.9	100.8	102.1	102.0	101.4	105.2	118.2	101.9	100.9
	SD	14.6	19.9	16.3	17.1	14.8	18.4	25.7	17.0	14.6
Systolic BP mm Hg	n	204	523	985	677	506	213	5	3113	1504
	MV	133.1	133.3	133.7	134.7	134.9	136.3	144.4	134.2	133.7
	SD	15.8	15.6	15.7	14.6	15.3	15.9	7.4	15.4	14.0
Diastolic BP mm Hg	n	204	523	985	677	506	213	5	3113	1504
	MV	84.9	84.8	84.7	85.3	86.2	87.2	90.0	85.3	86.3
	SD	10.6	11.1	10.8	10.4	11.2	10.4	4.6	10.8	9.9
Risk score	n	204	523	985	677	506	213	5	3113	1504
	MV	5.5	9.4	12.6	15.9	21.7	26.8	11.7	14.8	4.9
	SD	6.0	12.7	15.4	18.8	25.3	35.0	10.7	19.9	6.2

TABLE 21 Risk variables in men who never smoked cigarettes, and in cigarette smokers by number of cigarettes. Age 45–49. Main group

Number of cigarettes per day

		1–4	5–9	10–14	15–19	20–24	25	Unanw.	Total	Never cigarette smokers
Cholesterol mg/dl		299	742	1191	703	508	223	3	3669	1377
	MV	262.0	270.2	277.8	274.5	278.3	276.7	256.0	274.3	265.1
	SD	45.0	46.4	47.9	47.8	49.9	46.5	1.7	47.8	47.8
Triglycerides mM/l	n	299	742	1191	703	508	223	3	3669	1377
	MV	2.14	2.23	2.40	2.32	2.43	2.52	2.66	2.34	2.09
	SD	1.19	1.08	1.43	1.10	1.30	1.51	1.20	[illegible]	1.04
Glucose mg/dl		299	742	1190	703	508	223	3	3668	1377
	MV	101.2	100.8	102.4	103.9	106.0	107.6	91.3	103.1	101.6
	SD	21.0	15.5	21.0	17.5	25.8	29.9	9.5	20.9	20.2
Systolic BP mm Hg		299	742	1191	702	508	223	3	3668	1377
	MV	134.4	134.7	135.5	135.4	136.7	137.8	131.3	135.5	135.5
	SD	16	16.0	16.8	16.6	17.0	16.9	14.0	16.6	16.2
Diastolic BP mm Hg	n	299	742	1191	702	508	223	3	3668	1377
	MV	85.0	85.9	86.2	85.9	87.4	88.3	82.0	86.3	87.5
	SD	11.0	10.8	10.9	10.8	10.7	10.2	7.2	10.7	10.6
Risk score	n	299	742	1191	703	508	223	3	3669	1377
	MV	6.3	9.0	14.7	18.5	25.2	29.9	3.6	15.9	6.1
	SD	7.8	9.0	17.2	21.3	29.9	28.1	1.4	20.4	6.7

TABLE 22. Risk variables in relation to tobacco consumption in pure pipe smokers, in pure cigar smokers and in combined pipe–cigar smokers as compared with never-smoked-cigarettes.
Age 40–49 Main group

		Cigars		Total	Tobacco consumption (g/week) in pure pipe smokers.			Never smoked cigarettes
		Pure	Combined		<25 g	25–100 g	≥100 g	
Cholesterol mg/dl	n	239	344	1261	115	945	96	1377
	MV	265.3	263.5	266.3	263.4	267.6	264.9	265.1
	SD	43.9	45.3	49.0	54.9	49.2	40.0	47.8
Triglycerides mM/L	n	239	344	1261	115	945	96	1377
	MV	2.2	2.2	2.2	2.0	2.2	2.2	2.1
	SD	1.0	1.0	1.6	1.0	1.8	1.2	1.0
Glucose mg/dl		239	344	1261	115	945	96	1377
	MV	102.2	101.8	102.3	102.7	102.1	104.9	101.6
	SD	17.4	16.9	16.5	16.3	16.8	14.5	20.2
Systolic BP mm Hg	n	239	344	1261	115	945	96	1377
	MV	133.0	133.4	134.7	135.2	134.3	137.9	135.5
	SD	14.3	14.5	16.0	16.2	16.2	14.5	16.2
Diastolic mm Hg	n	239	344	1261	115	945	96	1377
	MV	86.2	86.7	85.5	86.6	85.0	87.1	87.5
	SD	10.1	10.3	10.6	10.2	10.5	11.4	10.6

TABLE 23 Serum cholesterol in relation to duration of cigarette smoking.
Daily cigarette smokers. Main group.

Years smoked / Age		0–5	5–15	15–25	25+	Unansw.	Total
		32	252	2033	778	19	3114
40–44	MV	259.5	265.8	268.4	273.1	257.1	269.2
	SD	61.5	45.8	50.4	55.2	42.9	51.4
		48	198	1046	2375	2	3669
45–49	MV	256.8	268.1	277.4	273.9	270.5	274.4
	SD	37.9	50.8	48.0	47.5	30.4	47.8
		80	450	3079	3153	21	6783
40–49	MV	257.9	266.8	271.5	273.7	258.4	272.0
	SD	48.4	48.0	49.8	49.5	41.5	49.6

TABLE 24 Seasonal variations of main risk variables. Age 40–49 Main group.

		Dec. – 72	June – 73
Cholesterol mg/dl	n	743	1233
	MV	273,3	255 7
	SD	47,8	45 1
Triglycerides mM/l	n	742	1233
	MV	2,33	2 17
	SD	1 18	1,25
Glucose mg/dl	n	742	1233
	MV	105,0	100,6
	SD	20,9	14 7
Systolic BP mm Hg	n	743	1233
	MV	136,5	133,9
	SD	16,3	15,3
Diastolic BP mm Hg	n	743	1233
	MV	87 1	86,2
	SD	10,6	10,7
Risk score	n	743	1233
	MV	11,9	8,4
	SD	20,3	11 7

TABLE 25 Seasonal variations in physical activity and feeling of stress Main group Age 40–49 (for definitions see tables 3 4,5)

		June 72		Dec 72		June – 73	
		n	%	n	%	n	%
Physical activity at work	Sedentary	279	58.0	382	51.4	596	48.3
	Moderate	120	24 9	187	25.2	375	30 4
	Intermediate	67	13.9	138	18 6	210	17.0
	Great activity	13	2.7	31	4.2	43	3.5
	Unanswered	2	0.5	5	0.6	9	0.8
Physical activity at leisure	Sedentary	91	18 9	182	24.5	226	18.3
	Moderate	281	58 4	445	59.8	735	59.6
	Intermediate	97	20.2	98	13.2	249	20.2
	Great activity	11	2.3	15	2.0	23	1.9
	Unanswered	1	0.2	3	0.3	0	
Subjective feeling of stress	Psychic tension	128	26.6	214	28.8	330	26.8
	High stress	262	54 5	425	57.2	691	56.0
	Press on deadlines	215	44 7	390	52.5	639	51.8
	Contented peaceable	151	31 4	189	25 4	314	25.5
	Unanswered	1	0 2	1	0 1	0	0 0

TABLE 26 Correlation coefficients (r) and their t-values for significance between some risk variables. Main group Age 40–44 and 45–49

Age		Chol./ Trl	Chol./ Gluc.	Chol./ S.BP	Chol./ D.BP	Chol / Weight	Chol./ $H/\sqrt[3]{W}$	Trl/ Gluc.	Trl/ S.BP	Trl/ D.BP	Trl/ Score	Trl/ Weight	Trl/ $H/\sqrt[3]{W}$	Gluc./ S.BP
	n	6910	6910	6910	6910	6853	6853	6910	6910	6910	6010	6853	6853	6910
40–44		0.41	0.07	0.16	0.16	0.12	–0.19	0.10	0.14	0.14	0.31	0.24	0.28	0.23
	|t|	37 4	5.8	13.5	13.5	10.0	16.0	8.4	11.8	11.8	27.1	21.2	24.2	19.6
	n	7906	7906	7906	7906	7844	7844	7906	7906	7906	7906	7844	7844	7906
45–49	r	0.34	0.04	0.12	0.11	0.09	–0.16	0.13	0.13	0 12	0.25	0.21	–0.27	0.20
	|t|	32.1	3.6	10 7	9.8	8.0	14.2	11 7	11 7	10.7	23.0	19 1	24.9	18 1
		14816	14816	14816	14816	14697	14697	14816	14816	14816	14816	14697	14697	14816
40–49		0.37	0.05	0 14	0 14	0.10	–0.18	0.12	0.13	0.13	0.28	0.22	–0.27	0.21
	|t|	48 4	6.1	17.2	17.2	12.2	22.3	14 7	16.0	16.0	35.5	27.5	34 1	26.1

Age		Gluc./ D.BP	Gluc./ Score	Gluc./ Weight	Gluc / $H/\sqrt[3]{W}$	S.BP/ D.BP	S.BP./ Weight	S.BP./ $H/\sqrt[3]{W}$	D.BP./ Score	D.BP./ Weight	D.BP./ $H/\sqrt[3]{W}$	Score/ Weight	Score/ $H/\sqrt[3]{W}$
		6910	6910	6853	6853	6910	6853	6853	6910	6853	6853	6853	6853
40–44		0 13	0.11	0.07	–0.11	0.70	0.29	–0.26	0.32	0.34	–0.32	0.15	–0.18
	|t|	10.9	9.2	5.8	9.2	81.5	25.2	22.4	28 1	30.1	28.1	12.6	15.2
	n	7906	7906	7844	7844	7906	7844	7844	7906	7844	7844	7844	7844
45–49		0 10	0 12	0.08	–0 10	0.71	0.23	–0.26	0.33	0.28	–0.29	0.14	–0.18
	|t|	8.9	10 7	7.1	8.9	89.6	21.0	23.9	31 1	25.9	26.9	12.6	16.3
		14816	14816	14697	14697	14816	14697	14697	14816	14697	14697	14697	14697
40–49		0.11	0.12	0.08	–0.10	0 70	0.26	–0.26	0.32	0.31	–0.30	0 14	–0.18
	|t|	13.5	14 7	9.8	12.2	119.3	32.8	32.8	41 1	39 7	38.3	17.2	22.3

The Oslo Study

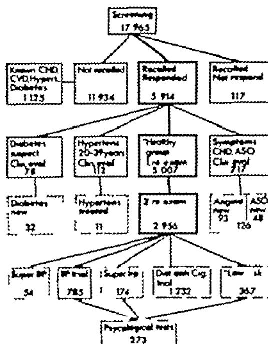


Figure 1 Oslo Study Flow hart of screening and follow-up studies.

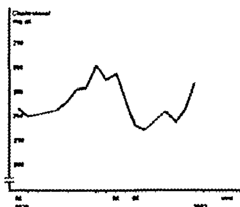


Figure 3 Oslo Study Seasonal variations of serum cholesterol. Age 40-49

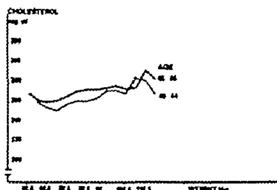


Figure 4 Oslo Study Serum cholesterol in relation to body weight

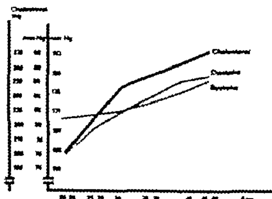


Figure 2. Oslo Study Serum cholesterol and blood pressure by age

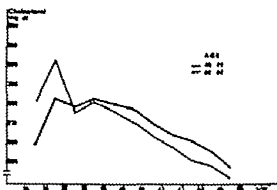


Figure 5 Oslo Study Serum cholesterol in relation to relative weight

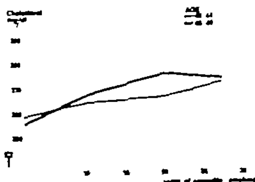


Figure 6. Oslo Study. Serum cholesterol in relation to duration of cigarette smoking

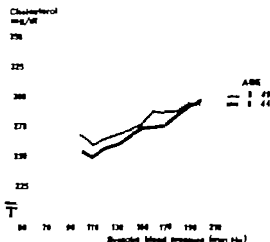


Figure 7. Oslo Study. Serum cholesterol in relation to systolic blood pressure.

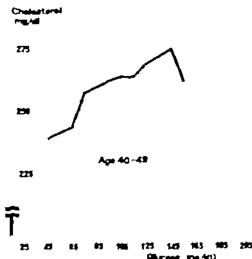


Figure 8. Oslo Study. Serum cholesterol in relation to blood glucose.

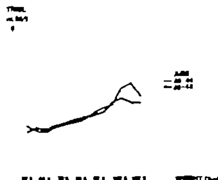


Figure 9. Oslo Study. Serum triglycerides in relation to body weight

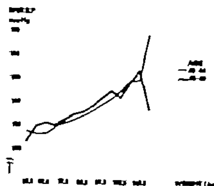


Figure 10. Oslo Study. Systolic blood pressure in relation to body weight.

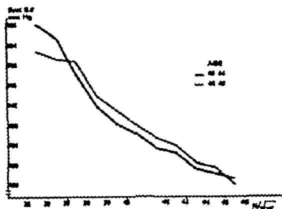


Figure 11 *Oslo Study Systolic blood pressure in relation to relative body weight*

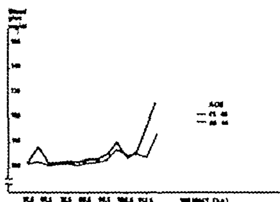


Figure 14 *Oslo Study Blood glucose in relation to body weight*

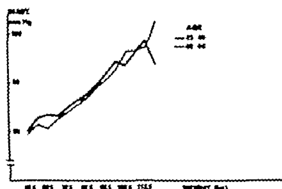


Figure 12 *Oslo Study Diastolic blood pressure in relation to body weight*

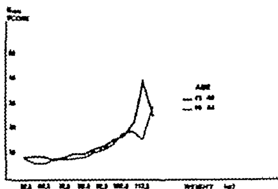


Figure 15 *Oslo Study Risk score in relation to body weight*

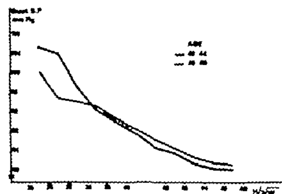


Figure 13 *Oslo Study Diastolic blood pressure in relation to relative body weight*

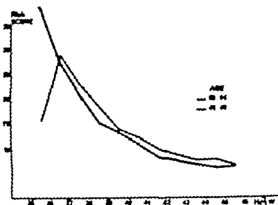


Figure 16 *Oslo Study Risk score in relation to relative body weight.*

IV Discussion

The attendance to the screening is low. Possibly there are several reasons for this. Firstly the men were asked to meet preferably within one week after the reception of the call letters. This may of course have been difficult to many. Secondly the examination was primarily a mass x-ray examination for tuberculosis which does not have the same strong appeal as it used to have. Lastly it was agreed that recall letters to non-attendants were not to be sent.

Always, when one hundred per cent attendance is not achieved, the possibility of selection exists which might weaken the representativity of the results. Whether such a selection has taken place is not known as yet. However this will be further studied in the planned linkage of the screening data to the 1970 Oslo census of socio-economic data such as education, type of work, income, housing facilities, and number of children.

The risk score model is based on mutual independance of the actual risk variables, which the present study has proved not always to be the case. This may weaken the validity of the risk score tables. Hopefully this will not be of any great importance.

Lack of physical activity is a marked feature in the mode of life in Western communities and a possible causal factor for the increase in atherosclerotic disease. In the present study about 50 per cent of the men had sedentary work, while only 3,6 % had real heavy manual work. However lack of activity during working hours seems to be compensated for during leisure time when only 21 per cent reported to be sedentary. A most interesting observation is the low risk profile of the work sedentary men with great physical activity at leisure. This holds for serum cholesterol, blood pressure and risk score (tables 11-18). Possibly these are health-conscious men with high educational status who compensate lacking possibilities for physical activity at work with great leisure activity.

Also in Oslo tobacco smoking is a most prevailing habit, and as many as 55,8 per cent of the men reported to be daily smokers. Cigarette smokers are by far most frequent (82,9 % of daily smokers).

It should be noticed that only 23,6 per cent of the daily cigarette smokers used filter cigarettes, while as many as one fourth of the men, aged 40-49 had quit cigarette smoking. Encouraging is the observation that 36 per cent of younger men (20-29) had never smoked cigarettes at all.

Correlation studies revealed a significant increase in the serum cholesterol value by increasing daily number of cigarettes, from 259 mg/dl in those smoking only 1-4 cigarettes a day to 276 and 273 in those smoking 20-24 and 25 or more cigarettes, respectively.

Interesting is also that the cholesterol value of those smoking only 1-4 cigarettes per day is the same as in those who had never smoked cigarettes.

Of great importance for the on-going discussion of CHD risk factors is also the observation that the serum cholesterol value increases with the duration of cigarette smoking. Thus those having smoked cigarettes for only 0-5 years have a cholesterol of 258 mg/dl as compared to 274 in those having been cigarette smokers for 25 years and more. The break down of these figures into age subgroups makes it unlikely that this cigarette effect on serum cholesterol is an effect of age only.

Also in the level of triglycerides, blood glucose, blood pressure and risk score there is an increasing trend with increasing number of cigarettes.

With regard to the level of the observed risk factors, it is again shown that those with atherosclerotic disease have higher values for

both cholesterol triglycerides blood pressure and risk score than the symptom-free «healthy» group

Also the blood glucose level is higher in the «atherosclerotic group» than in the «healthy» (table 8) The high glucose value in the hyper tonics is supposed to be due to the use of thia zides.

The comparison of epidemiological data from different geographical areas is of interest However the validity of such comparisons may be reduced when the analysis have been per formed in different laboratories. Nevertheless such comparisons may be useful

Of special interest are results from similar studies in other Scandinavian centers

The levels of serum cholesterol both in patients with CHD and in healthy men are very much the same as in earlier studies in Oslo (9 10) and in the Copenhagen study 1973 (7)

However the lipid levels are significantly higher than both in Gothenburg (16) Stock holm (5) and Uppsala (8)

On the other hand, the cholesterol level in Oslo is lower than in the county of Finn mark (2) which compares well with the choles terol value in North Karelia (13) It should be noticed that Finnmark in Norway and North Karelia in Finland top the list of CHD morta lity in their respective countries.

With regard to the triglycerides values it should be remembered that they are non fasting In the long-term follow-up studies it will be of special interest to study the CHD predictability of non-fasting triglycerides.

The blood pressure level is about the same in Oslo as was found in the other Scandinavian studies, and so is the prevalence of daily smok ers with the exception of Copenhagen where as many as 78 % of the 50-years old men were daily smokers and of the county of Finnmark and North Karelia

In view of possible preventive measures, the age dependance of CHD risk factors are of special interest. (table 10 and fig. 7) All the reported factors show increasing values with in creasing age However the most striking feature is the pronounced agegradient of serum chole sterol Values less than 200 mg/dl in age group 20-24 compare well with those found in certain developing countries where CHD is rare How ever unlike the situation in these countries, there is a steep increase of the cholesterol level from the age of 25 Indeed this is a most alarm ing observation It might however be an indi cation at what age preventive measures should be started to be most effective

Also serum triglycerides and blood pressure show increasing values with age These age gradients, however are not so pronounced as for cholesterol as shown in other studies.

The seasonal variation of serum cholesterol is marked being low during the summer and high during the winther months This varying cholesterol level is to be taken into considera tion when comparing values from different regions, and also when evaluating effect of intervention measures. The reasons for this seasonal variation of the cholesterol level are not known Variations in diet might partly be responsible

The positive correlation between serum cho- lesterol and cigarette smoking has already been commented.

Also in this study cholesterol triglycerides and body weight are intercorrelated

Of special interest is the observed correla tion between blood pressure and serum choles terol (fig 7 and table 26)

The intercorrelations of body weight triglycerides, blood pressure and blood glucose have also been demonstrated in other studies.

V Summary

The Oslo study is an epidemiological preventive investigation of CHD risk factors in Oslo men. All men aged 40-49 and a 7 per cent random sample of men aged 20-39 were mailed an invitation letter to meet at the Oslo Department of Health for a health examination which included a miniature x-ray of the lungs a non-fasting blood sample for total cholesterol triglycerides, and glucose measuring of height weight, and blood pressure The invitation letter included a questionnaire allowing the determination of known presence of coronary heart disease (CHD) cerebral vascular disease (CVD) hypertension atherosclerosis obliterans of the lower limbs (ASO), diabetes or symptoms of CHD and ASO

Tobacco smoking, physical activity at work and at leisure and subjective feeling of «stress» were also registered.

The study started May 1972 and the screening phase ended December 1973 During this period 17965 men or 62 1 % of the actual population were examined.

According to questionnaire answers the prevalence of disease were CHD 2,0 % ASO 0,2 % stroke 0,2 %, hypertension 2,5 % diabetes 0,8 % and other heart diseases 1 1 %

Sedentary work activity was reported in 49 % and heavy manual work in 3,6 % At leisure only 21 % were sedentary

High mental strain and press on dead lines were reported in 55,8 % and 50 4 % respectively while only 27.9 % reported to have given up high speed and ambitions in order to enjoy peaceful and quiet life

Daily smokers were 55,8 % of which 82,9 % were cigarette smokers At age 20-29 and 40-49 36,3 % and 18,9 % respectively had never smoked cigarettes.

The CHD risk variables were higher in those reporting atherosclerotic disease Thus, at age 40-49 cholesterol, triglycerides, blood pressure and risk score in CHD patients were 291.2 [illegible]77 140 4/91.9 and 17 4 respectively as compared with 267.2 [illegible].22 134.9/86.2 and 10.2 in the «healthy» group

The risk variables increased by age This was most pronounced with serum cholesterol which increased from 199 7 at age 20-24 to 70 4 at age 45-49

Men with great physical activity at work were characterized by high serum cholesterol, blood pressure and risk score while those sedentary at work had low risk variables. At leisure the opposite was found. Thus the most preferable risk profile was found in those with sedentary work and high physical activity at leisure

The number of daily cigarettes and the duration of cigarette smoking were correlated to the CHD risk variables, especially to serum cholesterol A sizeable seasonal variation in the level of risk variables was found, especially in cholesterol

Statistical analysis revealed intercorrelations between several risk variables. Serum cholesterol was correlated to triglycerides body weight and blood pressure Body weight was correlated to triglycerides blood pressure blood glucose and risk score

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The Statistical Institute of the Life Insurance Companies carried out the statistical analyses.

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Professor Knut Westlund took part in the forming of the study and advised in the statistical analysis of the results.

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VIII Addendum

TABLE A 1 Distribution of serum cholesterol (mg/dl) within 5-year age-groups. Main group

S chol. / Age		<125	125-149	150-174	175-199	200-209	210-219	220-229	230-239	240-249	250-259	260-269	270-279	280-289	290-299	300-309	310-319	320-329
20-24	n	3	18	79	114	33	29	22	26	12	10	9	6	7	1	2	1	1
	%	0.8	4.8	21.0	30.3	8.8	7.8	5.9	7.0	3.2	[illegible].7	2.4	1.6	1.8	0.3	0.5	0.3	0.3
25-29	n	1	10	52	1 4	53	57	47	42	42	31	35	19	18	6	1	6	5
	%	0.2	1.7	9.1	21.4	9.2	9.9	8.[illegible]	7.3	7.3	5.4	6.1	3.2	3.1	1.0	2.1	1.1	0.9
30-34	n	0	1	15	52	28	36	40	7	26	32	1	26	16	18	7	8	7
	%		0.3	4.0	13.8	7.4	9.5	10.6	7.1	6.8	8.5	5.6	6.8	4.2	4.8	1.9	2.1	1.9
35-39	n	0	3	12	35	21	25	36	40	37	34	23	26	25	16	12	13	6
	%		0.8	3.1	9.2	5.5	6.5	9.4	10.5	10.0	9.0	6.0	6.8	6.5	4.2	3.1	3.5	1.6
40-44	n	3	9	102	367	294	429	471	559	615	639	585	535	500	390	344	281	195
	%	0.0	0.1	1.5	5.3	4.3	6.2	6.8	8.1	9.0	9.2	8.5	7.7	7.3	5.6	5.0	4.2	2.8
45-49	n	1	12	84	282	258	349	489	584	664	698	707	711	610	502	473	360	288
	%	0.0	0.2	1.1	3.6	3.3	4.4	6.2	7.4	8.4	8.8	8.9	9.0	7.7	6.3	6.0	4.6	3.6

TABLE A 1 (cont.) Distribution of serum cholesterol (mg/dl) within 5-year age-groups. Main group.

S chol. / Age		330-339	340-349	350-359	360-369	370-379	380-389	390-399	400-424	425-449	450-474	475-499	500-524	525-549	550-574	575-599	600+	Total
20-24	n	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	376
	%	0.5																100
25-29	n	4	3	1	1	0	1	0	2	1	0	1	0	0	0	0	0	574
	%	0.7	0.5	0.2	0.2		0.2		0.4	0.2		0.2						100
30-34	n	3	1	3	3	3	3	0	0	2	0	0	0	0	0	0	0	378
	%	0.8	0.3	0.8	0.8	0.8	0.8			0.5								100
35-39	n	4	3	4	4	0	1	0	2	0	0	0	0	0	0	0	0	382
	%	1.0	0.8	1.0	1.0		0.3		0.5									100
40-44	n	143	115	98	92	39	30	16	30	6	11	4	0	0	2	0	5	6909
	%	2.1	1.7	1.4	1.3	0.6	0.4	0.2	0.4	0.1	0.[illegible]							100
45-49	n	242	151	1 4	9	6	41	42	47	13	9	2	0	0	1	0	3	7906
	%	3.1	1.9	1.6	1.2	0.8	0.5	0.5	0.6	0.2	0.1							100

TABLE A 2 Distribution of diastolic blood pressure (mm Hg) within 5-year age-groups. Main group.

Age	D.BP	<59	60-61	62 63	64-65	66-67	68-69	70-71	72 73	74-75	76-77	78-79	80-81	82 83	84-85	86-87	88-89	90-91	92 93
20-24		8	16	10	9	6	30	52	28	30	27	22	43	13	14	13	25	14	6
	%	2.1	4.3	2.6	2 4	1.6	8.0	13.8	7 4	8.0	7.2	5.9	11 4	3.5	3 7	3.5	6.6	3 7	1.6
25-29		6	11	6	5	8	31	55	27	31	45	49	94	20	38	27	34	35	8
	%	1.1	1.9	1 1	0.9	1 4	5 4	9.6	4 7	5 4	7.8	8.5	16 4	3.5	6.6	4 7	5.9	6.1	1 4
30-34	n	1	4	2	3	2	20	31	18	15	26	34	54	19	25	19	19	33	10
	%	0.3	1.1	0.5	0.8	0.5	5.3	8.2	4.8	4.0	6.8	9.0	14.3	5.0	6.6	5.0	5 0	8.7	2.6
35 39		0	2	1	4	4	13	23	12	16	22	28	52	15	26	16	30	47	8
	%		0.5	0.3	1.1	1.1	3 4	6.0	3.1	4.2	5.8	7.3	13.6	3 9	6.8	4.2	7.9	12.3	1 4
40-44		12	24	16	22	27	129	269	169	257	336	447	934	324	502	370	456	880	253
	%	0.2	0.3	0.2	0.3	0.4	2.0	4.0	2.4	3 7	5.0	6 4	13.5	4.6	7.2	5.3	6.6	12.7	3 7
45-49		16	16	15	37	21	123	282	145	258	339	477	1020	398	522	413	546	1045	326
	%	0.2	0.2	0.2	0.5	0.3	1.5	3.6	1.8	3.3	4.3	6.0	12.9	5.0	6.6	5.2	7.0	13.2	4 1

TABLE A 2 (cont.) Distribution of diastolic blood pressure (mm Hg) within 5-year age-groups. Main group.

Age	D. BP	94-95	96-97	98-99	100-101	102 103	104-105	106-107	108-109	110-111	112 113	114-115	116-117	118-119	120-121	122 123	124-125	126-127	128-129	130+	Total
20-24		5	1	0	3	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	376
	%	1.3	0.3		0 8			0.3													100
25-29	n	9	8	7	13	2	2	0	1	2	0	0	0	0	0	0	0	0	0	0	574
	%	1 6	1 4	1.2	2.3	0.3	0.3		0.2	0.3											100
30-34	n	12	6	9	8	0	1	0	2	4	1	0	0	0	0	0	0	0	0	0	378
	%	3.2	1.6	2.4	2.1		0.3		0.5	1 1	0.3										100
35-39		12	13	17	8	2	6	0	0	2	1	0	0	0	2	0	0	0	0	0	382
	%	3.1	3 4	4 4	2.1	0.5	1.6			0.5	0.3				0.5						100
40-44		261	208	16	333	66	89	36	78	83	20	19	8	22	23	4	3	2	5	7	6910
	%	3.9	3.0	3.2	4.9	1.0	1.3	0.5	1.1	1.2	0.3	0.3	0.1	0.3	0.3					0 1	100
45-49		344	235	301	421	89	102	42	96	149	25	22	7	15	25	7	3	5	3	16	7906
	%	4 4	3.0	3.8	5.3	1 1	1.3	0.5	1.3	2.0	0.3	0.3		0.2	0.3					0.2	100

1.

TABLE A 3 Distribution of systolic blood pressure (mm Hg) within 5-year age-groups. Main group

Age \ S. BP		<95	95-104	105-114	115-124	125-134	135-144	145-154	155-164	165-174	175-184	185-194	195-204	05-14	215-224	225-234	235+	Total
20- 4	n	0	4	35	109	121	76	23	6	2	0	0	0	0	0	0	0	376
	%		1 1	9.3	29.0	32.1	0.2	6 1	1.6	0.6								100
25-79	n	0	5	36	178	185	111	38	16	5	0	0	0	0	0	0	0	5 4
	%		0.9	6.3	31.0	32.2	19.3	6.6	.8	0.9								100
30-34	n	0	2	27	100	127	86	22	8	5	1	0	0	0	0	0	0	378
	%		0.5	7 1	26.5	33.6	22.8	5.8	2.1	1.3	0.3							100
35-39	n	0	4	21	99	107	92	35	16	6	1	1	0	0	0	0	0	382
	%		1.0	5.5	26.0	28.0	24.0	9 1	4.2	1.6	0.3	0.3						100
40-44	n	1	28	412	1507	2011	1600	764	370	120	49	22	17	5	0	2	1	6909
	%		0 4	6.0	21.8	29 1	23.2	11 1	5 4	1 7	0.7	0.3	0.3	0.1				100
45-49	n	4	45	448	1635	2137	1756	967	516	218	90	54	14	13	3	4	2	7906
	%		0.6	5 7	20.7	27.0	22.2	12.2	6.6	2.8	1 1	0.7	0.2	0.2				100

TABLE A 4 Distribution of serum triglycerides (mmol/l) within 5-year age-groups. Main group

Age \ Tri		<0 60	0.60-0 79	0.80-0.99	1.00-1 19	1.20-1.39	1 40-1.59	1.60-1 79	1.80-1.99	2.00-2.19	2.20-2.39	2.40-2.59	2.60-2.79	2.80-2.99
20-24			20	41	51	59	45	41	36	15	15	14	10	4
	%	0.5	5 3	10.9	13.6	15 7	11.9	10.9	9.6	3.9	3 9	3.6	2.6	1.9
25-29		2	19	52	84	83	59	67	39	38	25	26	14	15
	%	0 3	3.3	9 1	14.6	14.5	10.3	11 7	6.8	6.6	4.4	4.5	2.4	2 6
30-34	n	0	6	25	37	52	44	34	41	31	25	13	9	11
	%		1.6	6.6	9.8	13.8	11.6	9.0	10.8	8.2	6 6	3 4	2.5	3.0
35-39		0	4	19	35	42	41	39	45	31	21	23	13	13
	%		1.0	5.0	9.2	11 0	10.7	10 2	11.8	8.1	5.6	6.0	3 4	3 4
40-44	n	8	68	295	549	726	707	759	638	565	480	398	31	246
	%	0 1	1 0	4 2	8 0	10.5	10 2	11.0	9.2	8.2	7 0	5 7	4.5	3.6
45-49	n	2	58	283	538	751	884	814	754	687	591	489	370	89
	%	0 0	0 7	3.6	6 8	9 5	11.2	10.3	9.5	8 7	7 5	6 2	4 7	3.6

TABLE A 4 (cont.) Distribution of serum triglycerides (mmol/l) within 5-year age-groups. Main group

Age \ Trl		3.00-3.49	3.50-3.99	4.00-4.49	4.50-4.99	5.00-5.49	5.50-5.99	6.00-6.49	6.50-6.99	7.00-7.99	8.00-8.99	9.00-9.99	10+	Total
20-24	n	10	2	2	4	1	1	1	1	1	0	0	0	376
	%	2.6	0.5	0.5	1.0	0.2	0.2	0 2	0.2	0.2				100
25-29	n	26	6	6	5	4	0	1	0	1	1	1	0	574
	%	4.5	1.0	1.0	0.9	0.7		0.2		0.2	0.2	0.2		100
30-34	n	19	12	6	4	4	1	2	1	0	1	0	0	378
	%	5.0	3.2	1.6	1 1	1 1	0.2	0.5	0.2		0.2			100
35-39	n	12	15	14	2	4	0	2	2	4	0	0	1	382
	%	3 1	4.0	3 7	0.5	1.0		0.5	0.5	1.0			0.3	100
40-44	n	459	212	172	77	80	35	33	26	29	11	8	16	6909
	%	6.6	3.1	2.5	1 1	1.2	0.5	0.5	0.4	0.4	0.2	0.1	0.2	100
45-49	n	548	330	195	91	65	52	43	18	19	12	9	14	7906
	%	7.0	4.2	2.5	1 2	0.8	0.7	0.5	0.2	0.2	0.1	0.1	0.2	100

TABLE A 5 Distribution of serum glucose (mg/dl) within 5-year age-groups. Main group.

Age \ Gluc.		<55	55-59	60-64	65-69	70-74	75-79	80-84	85-89	90-94	95-99	100-104	105-109
20-24		0	1	3	3	5	23	47	54	73	54	47	23
	%		0.3	0.8	0.8	1.3	6.1	12.5	14.3	19 4	14.3	12.5	6 1
25-29	n	2	0	3	4	16	27	47	63	95	123	81	45
	%	0.3		0.5	0.7	2.8	4 7	8.2	11.0	16.6	21.5	4 1	7.8
30-34	n	0	1	1	7	6	6	27	51	63	65	58	36
	%		0.3	0.3	1 9	1.6	1.6	7.1	13.5	16 7	17.2	15.3	9.5
35-39		0	0	1	7	8	18	17	37	58	72	62	38
	%			0.3	1.9	2.1	4 7	4.6	9.8	15.2	18.9	16.2	10.0
40-44	n	4	8	12	38	69	158	350	598	944	1178	1080	889
	%	0.0	0.1	0.2	0.5	1.0	2.2	5.0	8.6	13 7	17.0	15.6	12.9
45-49		6	2	18	29	104	173	367	637	1101	1327	1240	943
	%	0 0	0.0	0.2	0.4	1.3	2.2	4.6	8.1	13.9	16.8	15 7	12.0

TABLE A 5 (cont.) Distribution of serum glucose(mg/dl) within 5-year age-groups. Main group

Age \ Gluc.		110 114	115 119	120 124	125 129	130 134	135 139	140 144	145 149	150 154	155 159	160+	Total
20-24	n	13	13	7	3	2	1	1	0	1	1	1	376
	%	3.5	3.5	1.8	0.8	0.5	0.3	0.3		0.3	0.3	0.3	100
25-29	n	25	10	10	8	5	3	1	0	2	0	4	574
	%	4.4	1.8	1.8	1.4	0.9	0.5	0.2		0.3		0.7	100
30-34	n	24	14	7	2	2	5	2	0	0	0	1	378
	%	6.3	3.7	1.9	0.5	0.5	1.3	0.5				0.3	100
35-39	n	23	12	9	6	4	3	2	0	2	0	3	382
	%	6.0	3.1	2.3	1.6	1.0	0.9	0.5		0.5		0.9	100
40-44	n	539	35	214	129	102	57	44	46	[illegible]	48	52	6909
	%	7.8	5.0	3.1	1.9	1.4	0.8	0.6	0.7	0.4	0.7	0.8	100
45-49	n	622	418	262	189	128	72	66	42	35	26	99	7906
	%	8.0	5.3	3.3	2.4	1.6	0.9	0.8	0.5	0.4	0.3	1.3	100

TABLE A 6 Distribution of risk score within 5-year age-groups. Main group

Age \ Risk score		1.00	1.01- 1.49	1.50- 1.99	2.00- 2.49	2.50- 2.99	3.00- 3.49	3.50- 3.99	4.00- 4.49	4.50- 4.99	5.00- 5.99	6.00- 6.99	7.00- 7.99	8.00- 8.99	9.00- 9.99	10.00- 11.49
20-24	n	64	59	53	56	39	20	13	9	8	13	17	6	3	3	5
	%	17.0	15.7	14.1	14.9	10.4	5.3	3.4	2.4	2.1	3.5	4.5	1.6	0.8	0.8	1.3
25-29	n	72	66	76	61	45	47	34	19	21	19	25	18	14	4	13
	%	12.5	11.5	13.2	10.6	7.8	8.2	6.0	3.3	3.7	3.3	4.4	3.1	2.4	0.7	[illegible]
30-34	n	20	34	37	36	35	29	19	14	20	27	[illegible]	10	10	9	9
	%	5.3	9.0	9.8	9.5	9.3	7.7	5.0	3.7	5.3	7.1	5.3	2.65	2.65	4	2.4
35-39	n	18	30	37	32	29	17	16	11	21	30	24	12	9	9	17
	%	4.7	7.9	9.7	8.4	7.5	4.5	[illegible]	2.9	5.5	7.9	6.3	3.1	[illegible]	2.4	4.5
40-44		115	288	443	471	508	452	411	394	301	489	376	325	290	[illegible]	[illegible]
	%	1.7	4.2	6.4	6.8	7.4	6.5	6.0	5.7	4.4	7.1	5.4	4.7	4.2	3.4	4.3
45-49	n	95	240	373	461	502	474	458	387	403	556	474	415	334	282	381
	%	1.2	3.0	4.7	5.8	6.4	6.0	5.8	4.9	5.1	7.0	6.0	5.3	4.2	3.6	4.8

TABLE A 6 (cont.) Distribution of risk score within 5-year age-groups. Main group.

Age \ Risk score		11.50-12.99	13.00-14.49	14.50-15.99	16.00-19.99	20.00-24.99	25.00-29.99	30.00-39.99	40.00-49.99	50.00-74.99	75.00-99.99	100.00-199.99	200.00-299.99	300.00-499.99	Total
20-24		1	1	2	1	2	0	0	1	0	0	0	0	0	376
	%	0.3	0.3	0.5	0.3	0.5			0.3						100
25-29		7	7	2	5	8	2	6	1	2	0	0	0	0	574
	%	1.2	1.2	0.3	0.9	1.4	0.3	1.0	0.2	0.3					100
30-34		11	10	5	3	8	3	3	2	4	0	0	0	0	378
	%	2.9	2.7	1.3	0.8	2.1	0.8	0.8	0.5	1.1					100
35-39		11	3	5	17	10	4	12	6	2	0	0	0	0	382
	%	2.9	0.8	1.3	4.4	2.5	1.0	3.1	1.6	0.5					100
40-44		229	173	145	247	224	126	148	81	88	31	20	4	1	6910
	%	3.3	2.5	2.1	3.6	3.2	1.8	2.1	1.2	1.3	0.4	0.3			100
45-49	n	273	230	200	366	286	203	223	104	111	34	39	4	0	7906
	%	3.5	3.0	2.5	4.6	3.6	2.6	2.8	1.3	1.4	0.4	0.5			100

TABLE A 7 Distribution of number of cigarettes and number of years smoked within 5-year age-groups.
Main group daily cigarette smokers.

Age \ No of cig.		1-4	5-9	10-14	15-19	20-24	25+	Unknown	Total
20-24		13	49	78	37	12	1	1	191
	%	6.8	25.7	40.8	19.4	6.3	0.5	0.5	100
25-29		20	49	90	60	24	5	1	249
	%	8.0	19.7	36.1	24.1	9.7	2.0	0.4	100
30-34		11	24	52	45	27	7	1	167
	%	6.5	14.4	31.1	27.0	16.2	4.2	0.6	100
35-39		11	31	44	47	25	22	1	181
	%	6.1	17.1	24.3	26.0	13.8	12.2	0.5	100
40-44		204	523	985	677	506	213	6	3113
	%	6.6	16.8	31.6	21.8	16.2	6.8	0.2	100
45-49		299	74	1191	703	508	223	3	3669
	%	8.1	20.2	32.5	19.2	13.8	6.1	0.1	100

Age \ No of years		5	5-14	15-24	25+	Unknown	Total
20-24		98	88	0	0	5	191
	%	51.3	46.1			2.6	100
25-29		31	214	3	0	1	249
	%	12.4	86.0	1.[illegible]		0.4	100
30-34		1	113	53	0	0	167
	%	0.6	67.7	31.7			100
35-39		2	37	132	9	1	181
	%	1.1	20.4	73.0	5.0	0.5	100
40-44		32	252	2033	778	18	3113
	%	1.0	8.1	65.3	25.0	0.6	100
45-49		48	198	1046	2375	2	3669
	%	1.3	5.4	28.5	64.7	0.1	100

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Lesions of the Legs in Diabetics

and
in patients with familial amyloidosis and polyneuropathy

By Folke Lithner

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Abstract

A number of skin lesions localized to the lower extremities, mainly in elderly patients are described, one lesion consisted of areas of erythema, the size of a child's palm or larger with or without necrosis, and another of purpura and pigmentation. The areas of erythema, purpura and pigmentation were localized to the legs as well as to the feet. A third lesion was yellow toe nails. These lesions are common and have a characteristic appearance. They were observed in patients with open diabetes and also in those not known to have diabetes. The latter group of patients had diabetic glucose tolerance curves. These patients may also have skeletal destructions in the feet. The skin lesions were also seen in younger diabetics, but only in those with long duration of the disease. Cutaneous erythema, with or without necrosis, most often heals, but may recur. Purpura may sometimes be transformed to pronounced, uniform, dark brown pigmentation of the legs.

Precipitating factors of these skin lesions could usually be established, most often being cardiac decompensation with or without edema of the legs or edema of the legs of other cause. The connection in time between precipitating factors and skin lesions was obvious. It is suggested that the skin lesions are due to an altered mode of reaction to these precipitating factors in diabetics or certain diabetics.

Roentgenologically demonstrable skeletal destructions in the feet were more common in diabetics with erythema, with or without necrosis, of the feet, more especially in those with erythema and necrosis than in diabetic patients without these skin lesions. A higher frequency of precipitating factors of the skin lesions was seen in patients with skeletal destructions than in those without. The skeletal destructions and cutaneous necrosis are supposed to be equivalent lesions localized to different tissues in the feet. When comparing patients with skin lesions of the feet having the form of distal gangrene to patients with cutaneous erythema and necrosis of the feet, excluding distal gangrene, no difference between the two groups of patients with respect to age, duration of diabetes, occurrence of precipitating factors and the occurrence of skeletal destructions could be demonstrated. No distinction between areas of cutaneous erythema with necrosis and distal diabetic gangrene could be demonstrated. In our experience distal diabetic gangrene is always surrounded by a reddened border zone. The areas of cutaneous erythema without necrosis are understood to be incipient diabetic gangrenes.

In a number of patients with familial amyloidosis and polyneuropathy several types of skin lesions localized to the lower extremities are described. These skin lesions had an apparent resemblance to skin lesions in diabetics. Skeletal destructions in the feet of these patients were also seen. None of the patients was diabetic.

Cutaneous reactions of the extremities to local thermal trauma were studied in diabetics and in patients with familial amyloidosis and polyneuropathy. Purpura within the area of traumatization was often seen, especially in the lower extremities. Purpura was more often seen and was more abundant in diabetics than in controls. This was true especially in patients with juvenile diabetes of long duration and in patients with maturity-onset diabetes. Diabetics with atrophic circumscribed skin lesions (Melin) in the lower extremities nearly always had purpura within the area of trau-

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UMEÅ 1976

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II Lithner F Cutaneous reactions of the extremities of diabetics to local thermal trauma. Acta med. scand 198 319 1975

III. Lithner F & Hägg, E.: Cutaneous reactions of alloxan diabetic rats to local thermal trauma. Upsala J Med. Sci. 80 99 1975

IV Lithner F Skin lesions of the legs and feet and skeletal lesions of the feet in familial amyloidosis with polyneuropathy Acta med scand. In press.

V Lithner F: Purpura, pigmentation and yellow nails of the lower extremities in diabetics. Acta med. scand. In press.

VI. Lithner F & Hietala, S.-O: Skeletal lesions of the feet in diabetics and their relationship to cutaneous erythema with or without necrosis on the feet. Acta med. scand. In press.

These papers will be referred to by their Roman numerals.

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Contents

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Abstract

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Precipitating factors of these skin lesions could usually be established, most often being cardiac decompensation with or without edema of the legs or edema of the legs of other cause. The connection in time between precipitating factors and skin lesions was obvious. It is suggested that the skin lesions are due to an altered mode of reaction to these precipitating factors in diabetics or certain diabetics.

Roentgenologically demonstrable skeletal destructions in the feet were more common in diabetics with erythema, with or without necrosis, of the feet, more especially in those with erythema and necrosis than in diabetic patients without these skin lesions. A higher frequency of precipitating factors of the skin lesions was seen in patients with skeletal destructions than in those without. The skeletal destructions and cutaneous necrosis are supposed to be equivalent lesions localized to different tissues in the feet. When comparing patients with skin lesions of the feet having the form of distal gangrene to patients with cutaneous erythema and necrosis of the feet, excluding distal gangrene, no difference between the two groups of patients with respect to age, duration of diabetes, occurrence of precipitating factors and the occurrence of skeletal destructions could be demonstrated. No distinction between areas of cutaneous erythema with necrosis and distal diabetic gangrene could be demonstrated. In our experience distal diabetic gangrene is always surrounded by a reddened border zone. The areas of cutaneous erythema without necrosis are understood to be incipient diabetic gangrenes.

In a number of patients with familial amyloidosis and polyneuropathy several types of skin lesions localized to the lower extremities are described. These skin lesions had an apparent resemblance to skin lesions in diabetics. Skeletal destructions in the feet of these patients were also seen. None of the patients was diabetic.

Cutaneous reactions of the extremities to local thermal trauma were studied in diabetics and in patients with familial amyloidosis and polyneuropathy. Purpura within the area of traumatization was often seen, especially in the lower extremities. Purpura was more often seen and was more abundant in diabetics than in controls. This was true especially in patients with juvenile diabetes of long duration and in patients with maturity-onset diabetes. Diabetics with atrophic circumscribed skin lesions (Melin) in the lower extremities nearly always had purpura within the area of trau-

matization and often had an intensely reddened border round this area of traumatization. They eventually developed atrophic circumscribed skin lesions on the area of traumatization on the legs. In patients with familial amyloidosis and polyneuropathy purpura also was seen within the area of traumatization more often than in the controls. In addition some of these patients also developed atrophic circumscribed skin lesions at the sites of traumatization. Cutaneous reactions to local thermal trauma were investigated in alloxan diabetic rats too. Especially long term alloxan diabetic rats had a pronounced reaction.

The altered reaction in diabetics is related to microangiopathy and polyneuropathy and, in patients with familial amyloidosis and polyneuropathy to polyneuropathy and possibly to amyloid deposits in the walls of the small blood vessels. The occurrence of lesions in the small blood vessels as well as peripheral neuropathy with impaired capacity to vary the blood circulation distally in the lower extremities will probably impede the passage of oxygen from the capillary blood to tissue cells as well as the passage of metabolites in the opposite direction

Introduction

Lesions, especially skin lesions, on the lower extremities of diabetics have been studied at the Department of Medicine in Umeå for more than 15 years. In 1964 Melin (45) described an atrophic circumscribed skin lesion in the lower extremities of diabetics. The present author has described (I V), especially in elderly diabetics, some lesions, apparently typical of diabetes and which have not been described previously or have not been associated with diabetes. These are.

a) Areas of cutaneous erythema, with or without necrosis, localized to the legs and feet.

b) Purpura and pigmentation localized to the lower extremities.

c) Yellow toe nails.

It was apparent that these lesions usually were precipitated by certain obvious factors, predominantly cardiac decompensation with or without edema of the legs. We suggested that this reflects an altered reaction *in diabetics or certain diabetics to* these precipitating factors.

The initial stages of atrophic circumscribed skin lesions (Melin) which have received little attention (23), have also been studied (I)

Destruction and demineralization of the bones of the feet in diabetics are well-known. The possible connection between these skeletal lesions and cutaneous lesions in the form of erythema, with or without necrosis, have been studied, as has that between so-called *distal diabetic* gangrene and cutaneous erythema with or without necrosis (VI).

Familial amyloidosis with polyneuropathy has been studied at our Department of Medicine (2 7 32, IV) These patients often have similar cutaneous and other

lesions in the lower extremities to those of diabetics with long duration of their disease (IV).

The question of an altered reaction in diabetics was studied using thermal traumatization, both heat and cold, of the skin of the extremities of diabetics (II) A corresponding study was performed in patients with familial amyloidosis and polyneuropathy (IV) and alloxan diabetic rats (III). The investigation of alloxan diabetic rats was undertaken because in human diabetics it is not possible to determine the exact duration of the disease, especially in those with maturity-onset diabetes.

History

In 1964 Melin (45) described an atrophic circumscribed skin lesion in the lower extremities of diabetics. Melin demonstrated that these lesions not are stationary These skin changes disappear after one or more years, and meanwhile new ones appear the picture as a whole remaining unchanged. In a microangiographic study and disappearance studies with locally injected isotopes, the atrophic skin lesions were more abundantly vascularized than the adjacent intact skin. Melin's description has been confirmed by a number of authors. These skin lesions are now usually called dermopathia diabetica (cf I)

The skin changes described by Melin are to be found in young patients with juvenile diabetes as well as in patients with maturity-onset diabetes. The occurrence increases with increasing duration of diabetes and is also correlated to the incidence if peripheral polyneuropathy of the lower extremities.

As is known, the occurrence of diabetic microangiopathy is correlated to the duration of the disease (28 39 53 54). Diabetic microangiopathy demonstrated as an increased width of the capillary basement membrane, occurs in the upper as well as in the lower extremities, though it is more pronounced in the latter (1 62). The capillary basement membrane is normally thicker in the lower than in the upper extremities even in non-diabetics (66) the thickness increasing with age (39) It has long been known that distal gangrene of the lower extremities is more common in diabetics (11). Diabetic gangrene is not only localized to the aera of the lower extremities but can also be localized to the dorsa of the feet (22, 36) and sometimes appears as patchy areas of the lower legs (25). There is no information as to whether these patchy areas are surrounded by cutaneous erythema or not (cf I). In most papers concerning diabetic gangrene (cf. VI) the gangrene was considered to be due to arterio- or arteriolosclerotic occlusion of the arterial lumen. There is, however no convincing evidence for this hypothesis (16 67) Furthermore, it is controversial whether arterial intimal lesions are more common among diabetics than among non-diabetics (13 26, 50). Diabetics have often been reported to have good pulses, in spite of the presence of gangrene of the toes,

in comparison with non-diabetics with atherosclerotic vascular foot lesions, who usually have intermittent claudiocation and absence of pulses in the popliteal artery and the posterior tibial artery (46)

Goldenberg and associates (25) called attention to what they described as prolifer ative changes in the endothelium of the arterioles they considered these lesions to be the cause of diabetic gangrene. Other authors (63 67) question whether or not they are of any special significance in the pathology of diabetic vascular disease and whether or not they are of an occlusive nature.

In investigations of the circulation in the lower limbs of diabetics (15 37 48 51) no obvious effect on the blood supply to the legs of diabetics with disease of long duration was found, except possibly in connection with maximal muscular work. This was true in young patients with juvenile diabetes of long duration as well as in patients with maturity-onset diabetes. The presence of peripheral polyneuropathy in diabetics, however impairs the capacity to vary the blood circulation to the skin area in question (24 47) The importance of diabetic neuropathy for the development of diabetic gangrene is an open question, as is the importance of diabetic microangiopathy The pathogenesis of diabetic gangrene is still unknown (16).

Cutaneous erythema in connection with diabetes is well known. Reddening of the face in diabetics was first described by von Norden and Isaak, who termed it rubeosis facies (52) Lundbaek (42) has described a condition which he calls rubeosis plantarum. Ipsen (34) mentions that in warm gangrene the border of the necrotic area may be reddened. Most of his patients were diabetics. In the literature no attention has, however been paid to the observations of Ipsen. The occurrence of cutaneous erythema surrounding diabetic gangrene has, to our knowledge, never been discussed (cf I)

In connection with the question as to whether areas of cutaneous erythema, with or without necrosis, localized to the legs and feet in diabetics, have been described earlier it should be mentioned that cutaneous erythema of the leg in connection with peripheral arterial ischaemia has been described (41) but that information as to the possible concomitant presence of diabetes is lacking. Ischaemic ulcers of the legs (19 20, 33) and so-called hypertensive ulcers (44) of the legs have also been described without mention of whether diabetes was present or not.

Purpura, especially localized to the lower extremities, has earlier been given different names, for example, the purpura of Schamberg, Gougerot and Blum or Majochi (cf 38) Increased capillary fragility is said to characterize these conditions. Information as to a possible relationship to diabetes is lacking. It is, however well known that diabetics have increased capillary fragility which was probably first described by Hanum (29). In a survey article (17) diabetes is mentioned as a possible cause of purpura but no information about the localization of the purpura was given. To our knowledge, no systematic studies or case reports have yet been published. Cardiac decompensation has also been mentioned as a possible cause of purpura (18) but again no systematic studies have been reported and no mention has been made of a possible connection with diabetes.

Yellow nails have earlier been described in patients with peripheral vascular disease (30, 58) and considered a symptom of impaired peripheral blood supply Yellow nails have also been described in connection with primary lymphedema (31 59) and cardiac decompensation (60) To our know

ledge, a connection with diabetes has not been described earlier

In diabetics, destructions of the bones of the foot under intact skin or without communication to skin ulcerations were probably first mentioned by Jordan in 1936 (35) Skeletal destructions in the feet of diabetics have been attributed to neuropathy by a number of authors (cf I). Although such patients with skeletal destructions always have neuropathy (12) there is no convincing evidence that the latter is the cause of the skeletal lesions (55 56) Erythema and other skin lesions localized to the feet concomitantly with Charcot joints in diabetics have been described (43 61)

Skin changes in familial amyloidosis and polyneuropathy have received little attention. Andrade (8) mentioned perforating trophic ulcers and Andersson and Bjerle (3) reported trophic changes, cutaneous ulcerations and peripheral reddish colouring of the extremities. These patients with amyloidosis have, as have patients with diabetes, a decreased capacity to vary the blood perfusion distally in the lower extremities (3) There are amyloid deposits in the walls of the small blood vessels of these patients (5 32) They have not been observed to have any signs of occlusive arterial disease (3 IV)

Definitions, Material and Methods.
See I VI

Results

Lesions of the legs in diabetics

The lesions dealt with in this paper are classified as 1) Areas of cutaneous erythema, with or without necrosis, localized to the legs and feet 2) Purpura and pigmentation on the lower extremities 3) Yellow toe nails 4) The initial lesions in atrophic circumscribed skin lesions of the lower extremities (Melin) 5) Skeletal lesions of the feet 6) Distal diabetic gangrene.

1) Areas of cutaneous erythema with or without necrosis

On the legs and/or feet in 81 patients, especially elderly diabetics, there were observed cutaneous lesions consisting of areas of erythema the size of a child's palm or larger (I) There was often edema in the erythema and this edema extended outside the reddened area. The center of the erythema was often necrotic. The necrosis involved all layers of the skin and was nearly always painless. The erythematous area was always many times larger than the area of necrosis. The necrosis usually began as a large zone of erythema in which central necrosis gradually developed. In a number of patients who also had edema of the legs it was possible to follow the course of events. The swelling always preceded the development of erythema, and the latter receded when the swelling subsided. These skin lesions usually appeared within a few days, and were usually reversible. It is characteristic that the lesions may recur after once having healed. The lesions were seen in patients with open diabetes as well as in patients not known to have diabetes.

The patients with erythema and necrosis more often had open diabetes than those with erythema alone. The former group

differed also from the latter in having a significantly longer duration of diabetes. In young patients these lesions were only seen in those with diabetes of long duration (II) About 53 % of the men and about 76 % of the women with such skin lesions had open diabetes.

On the basis of appearance alone, it may be difficult to distinguish the skin lesions described here—cutaneous erythema and erythema accompanied by necrosis—from erysipelas of the leg. However the patients with the described skin lesions usually are afebrile and have neither increased ESR nor leucocytosis. Such findings, when present, have always been attributed to another acute disease. In doubtful cases AST (antistreptolysin titre) has been determined the values have always been normal. Patients with erysipelas on the legs and feet always had elevated AST (I, V)

2) Purpura and pigmentation

More or less widespread, usually bilateral, purpura was observed on the lower extremities of 52 patients, mainly elderly diabetics (V) Erythema with or without necrosis was found in most patients concomitantly on the same extremity Most often the purpuric lesions were observed within the erythematous areas. Petechiae were transformed to small, pigmented, non atrophic spots. These latter pigmented spots were often found on the lower extremities concomitantly with the above-mentioned purpuric lesions. Like cutaneous erythema purpura was observed in patients with open diabetes as well as in patients not known to have diabetes.

Pigmentation alone was observed in 51 patients, mainly elderly diabetics (V) In comparison to the atrophic circumscribed skin lesions described by Melin (45), these pigmented, non-atrophic spots do not appear as atrophic depressions in the skin surface. The pigmented, non-atrophic spots were often confluent, forming larger areas. The degree of pigmentation varied from numerous, light brown, small spots to a pronounced dark brown pigmentation of the dorsal parts of the feet and the legs as well as the distal parts of the thighs. This latter type of pigmentation was seen in a few diabetics with severe and recurrent *cardiac decompensation of several years* duration.

In young patients purpura and pigmentation were only seen in those with diabetes of long duration (V)

3) Yellow toe nails

Smooth, somewhat thickened toe nails of yellow or yellowish-green colour were observed in 45 patients, mainly elderly diabetics. One diabetic with severe cardiac decompensation also had yellow finger nails. As a rule, all toe nails were affected, often most pronounced on the nails of the great toes, sometimes only on the distal part of the nails. Almost all patients had other lesions on the lower extremities — erythema, with or without necrosis, distal gangrene or purpura.

4) The initial lesions in atrophic circumscribed skin lesions of the lower extremities (Melin)

Superficial encrusted ulcers, the size of confetti or somewhat larger surrounded by a reddened border were observed in 8 diabetics (I) The ulcers were located on the anterior aspect of the legs, they were multiple and arranged in a linear pattern or in groups. In the patients in which it was possible to follow the clinical course, the described lesions gradually developed into the lesions described by Melin (45)

5) Skeletal lesions of the feet

Roentgenological examinations of the feet were performed in 70 patients with cutaneous erythema, with or without necrosis, on the feet (VI) Sixty five had open diabetes and four had diabetic glucose

tolerance curves. Twenty-seven of the 70 patients had roentgenologically demonstrable destructions in the bones of the feet. These 70 patients were compared to 61 diabetic control patients of corresponding age and duration of diabetes but without these skin lesions in the feet. Only four of the 61 control patients had destructions in the bones of the feet and all these destructions were small. Patients with skeletal destruction in the feet usually had demineralization of the bones of the feet as well. Only demineralization of the bones of the feet was observed more often in patients with these skin lesions than in patients without.

There were no significant difference in the occurrence of neuropathy in patients with skin lesions of the feet compared to diabetic control patients, nor was there any difference in the occurrence of neuropathy in patients with skin lesions and skeletal destructions compared to patients with skin lesions with no skeletal destructions.

Regression of skeletal destructions was observed in five of 14 investigated patients, two of whom showed almost complete reconstruction of previously destroyed bones.

6) Distal diabetic gangrene

In this work distal gangrene refers to cutaneous necrosis of the toe(s) or heel. In an earlier article (I) we have expressed the opinion that there is probably no clear distinction between cutaneous erythema, with or without necrosis, localized to the legs and feet and distal diabetic gangrene. For that reason it was of interest to compare patients with skin lesions in the form of distal gangrene to patients with cutaneous erythema with necrosis on the foot excluding distal gangrene. No difference between the two groups of patients with respect to age, duration of diabetes, occurrence of precipitating factors such as cardiac decompensation and the occurrence of skeletal destructions could be demonstrated (VI). The importance of precipitating factors to the development of among other things, cutaneous erythema, with or without necrosis, localized to the lower extremities, is dealt with later in this study.

Concerning the lesions on the lower extremities described as cutaneous erythema, with or without necrosis, purpura and pigmentation and yellow toe nails, respectively, they were seen in patients with open diabetes as well as in patients not known to have diabetes. However, comparison of the glucose tolerance test values for the patients with these lesions and not known to have diabetes with those of a control group (I) showed a significant difference in the diabetic direction for all the three groups. This was also true if the patients with cutaneous erythema, with or without necrosis, were divided into two groups, namely the group of erythema with necrosis and erythema without necrosis, and if the patients with purpura and pigmentation were divided into two groups, namely the group of purpura with or without pigmentation and the group with pigmentation only.

In a number of patients with erysipelas, purpura within the area of erysipelas was observed on the lower extremities. These patients had open diabetes or diabetic glucose tolerance. Patients with no purpura within the area of erysipelas generally had normal glucose tolerance.

Precipitating factors could generally be established for the lesions of the lower extremities described above. The precipitating factor was most often cardiac decompensation with or without edema of the legs, but also deep venous thrombosis of the leg, nephropathy with edema of the

legs or edema of the legs of unknown cause could also be precipitating factors. They may possibly be precipitated by peripheral arterial insufficiency

Cutaneous erythema, with or without necrosis, purpura and pigmentation were not only seen in elderly diabetics. They were also seen in younger diabetics with a long duration of the disease. The precipitating factor was then generally nephropathy with edema of the legs (II V) The connection in time between precipitating factors and the skin lesions, cutaneous erythema, with or without necrosis, and purpura, was obvious. As a rule, cutaneous erythema, with or without necrosis, disappeared when the precipitating factors subsided. Precipitating factors to the skin lesions were more common in patients not known to have diabetes compared to patients with open diabetes. Concerning skeletal destructions of the feet (VI), precipitating factors to the skin lesions of the feet in these patients generally could be established, the most common factor being cardiac decompensation Higher frequency of precipitating factors to the skin lesions was seen in patients with skeletal destructions than in those without.

Regression of skeletal destructions was seen in five of 14 investigated patients after immobilization or after treatment of the cardiac decompensation and local treatment consisting of elimination of edema of the lower legs and feet. Seven patients had progressive skeletal destructions of the bones of the feet and two had unaltered destructions. None of these seven patients received the treatment mentioned, only one of the latter two patients received the same treatment as the above-mentioned five patients with regression of skeletal destructions.

The time of the appearance of these skin lesions was known in most cases. They appeared within the course of a few days to a few weeks. The erythema most often disappeared after one to a few weeks. The necrosis healed far more slowly There are obvious reasons why the time of onset of the skeletal destructions could not be determined. They seem to develop within the course of a few weeks while regression of these skeletal destructions probably occurred far more slowly

Lesions of the legs in patients with familial amyloidosis and polyneuropathy

Twenty-one patients with this disease were examined at our Department of Medicine (IV) The patients often had characteristic skin lesions localized to the lower legs and feet. The lesions were classified as atrophic skin lesions, hypertrophic scar like lesions, rubeosis plantarum, spontaneous blisters, necrotic skin lesions, yellow nails, traumatic skin lesions, purpura and pigmentation. Skeletal destructions in the feet were also demonstrated in three of these patients.

In many respects these lesions of the lower extremities are similar to those of long term juvenile diabetes and maturity onset diabetes.

Cutaneous reaction to local thermal trauma of the extremities of diabetics and patients with familial amyloidosis and polyneuropathy

Diabetics.

Traumatization with local heat or cold to the skin of legs and forearms was performed in 35 diabetics and 25 controls (II) The diabetics were of varying age and had varying duration of diabetes. Most of them had atrophic circumscribed skin lesions (Melin) in the lower extremities. The temperature used at traumatization with heat was 60 or 55 C. The majority of the subjects investigated developed erythema and blisters at the sites of thermal trauma and none displayed necrosis of the

skin. Many of those investigated developed small haemorrhages (petechiae) in the area of traumatization. A number of the diabetics developed an intensely reddened border surrounding the area of traumatization. A large number eventually developed atrophic circumscribed skin lesions at the sites of traumatization. Observation 24 hours after the experimental trauma disclosed a blister an erythema or no reaction at all. If a blister was present, it was often impossible to determine whether or not an erythema had developed as well because the blisters were often opaque. Consequently no information is given about the presence or not of erythema.

On the areas traumatized with either heat or cold, petechiae were observed in both diabetics and controls. Petechiae were often first observed 7 days after traumatization and thus appeared later than did the blisters. Petechiae were observed approximately as often at sites traumatized with heat at 60°C as at those traumatized with cold. They were seen less often at sites traumatized with heat at 55° C. Petechiae occurred more often and were more abundant on the legs than on the forearms. Petechiae were observed more often in the diabetics than in the controls. Among the controls, petechiae were only observed in those over 50 years of age, only on the legs and only after traumatization with heat at 60 C or with cold, and not after traumatization with heat at 55°C. The petechiae in controls were always solitary with one exception, a man of 69 who had abundant petechiae at the site of traumatization both with heat at 60°C and with cold. This person had a clearly diabetic type of glucose tolerance test.

The occurrence of petechiae was significantly more common among diabetics under 50 years of age compared to controls of corresponding age (legs, heat and cold). The difference between diabetics over 50 and corresponding controls was significant with heat at 55°C but not with heat at 60 C or with cold. In young diabetics the occurrence of petechiae was related to the duration of diabetes, being significantly more common among those with long duration (legs, cold). In diabetics the petechiae were more abundant and were often confluent.

Many persons presented a zone of intense redness. This was never seen as early as 24 hours after the traumatization but was generally manifest by 7 days. This zone persisted for varying lengths of time from one week up to four months. It occurred only on the legs and only after traumatization with heat at 60°C or with cold. It was seen only in diabetics, young and old, and, among those, only in persons who had had the disease for ten years or more and who also had atrophic circumscribed skin lesions on the legs. This intensely reddened border resembled that seen in the initial lesions of atrophic circumscribed skin lesions (Melin)

Nineteen of the 35 diabetics had atrophic circumscribed skin lesions on the lower extremities and three on the upper extremities. After traumatization on the skin of the legs with heat at 60 C or with cold, 16 of these 19 patients developed atrophic circumscribed skin lesions on the area of traumatization on the legs. Only one diabetic without atrophic circumscribed skin lesions developed these lesions as result of the traumatization. The interval between traumatization and the appearance of these skin lesions was three months or more. As a rule, the lesions were pigmented and could not be distinguished from those observed in the patients with atrophic circumscribed skin lesions. None of the controls developed atrophic circumscribed

skin lesions as a result of the traumatization. All diabetics with an intensely reddened border round the area of traumatization developed atrophic circumscribed skin lesions. The three patients who already had atrophic circumscribed skin lesions on the forearms developed atrophic circumscribed skin lesions on the forearms as a result of the experimental traumatization with heat at 60°C and one of them developed such a lesion after traumatization with cold.

Patients with familial amyloidosis and polyneuropathy

An identical traumatization with local heat and cold was performed in 11 of these patients (IV). Petechiae were often seen within the area of traumatization in these patients too. Petechiae were observed more often in patients with amyloidosis than in controls. Petechiae occurred more often and were more abundant on the legs than on the forearms. In the patients they occurred after traumatization with heat at 60°C as well as at 55°C. In patients, the petechiae were more abundant and were often confluent. The occurrence of petechiae were significantly more frequent in patients younger than 50 years compared to controls of corresponding age. The difference in the occurrence of petechiae on the legs of patients older than 50 years and corresponding controls was not significant. The difference in the frequency on the forearms was significant. Nine of the 11 patients had atrophic skin lesions on the legs before the application of local thermal trauma. After thermal traumatization with heat at 60°C, four of these 11 patients developed atrophic circumscribed skin lesions as a result of the experimental trauma. Three of these four patients also developed similar lesions after traumatization with cold. The length of time between traumatization and appearance of these skin lesions was three months or more. The lesions were pigmented and were very much like the skin lesions these patients already had on the lower extremities.

In these patients as well as in the diabetics there were no obvious qualitative differences between the skin reactions to the local application of heat or cold.

Cutaneous reactions of alloxan diabetic rats to local thermal trauma

A motivation for this study was stated in the introduction. A further advantage with *experimental diabetes is that the* assessment of the induced lesions can be performed without any knowledge of the presence or absence of diabetes or the age of the animal. The skin of the external ears was traumatized. Only heat was used.

There generally occurred erythema or necrosis within the area of traumatization; there was no red, marginal zone around the lesions. The intensity of erythema and the extent of necrosis was semiquantitatively assessed by inspection. The skin was inspected 1, 7, 14 and 21 days subsequent to the traumatization. The compared animal groups differed from each other in one or more of the following three factors: age, diabetes of short duration and diabetes of long duration. Erythema was found to be more intense in young than in old controls after 1 and 7 days. Young diabetic rats had a more intense erythematous reaction than age-matched controls after 14 and 21 days. Long-term diabetic rats had a markedly increased skin redness at all times compared with controls of the same age. As to the extent of necrosis, there were no significant differences between the experimental groups, however there was a tendency for an increased amount in the long-term when compared with the short term diabetic rats.

Discussion

The connection between the skin lesions described and diabetes

As mentioned above, cutaneous erythema, with or without necrosis, purpura and pigmentation and yellow toe nails, respectively were observed in the lower extremities of diabetics, mainly elderly diabetics. They were also seen in elderly persons not known to have diabetes. However comparison of the glucose tolerance test values for these groups of patients with those of a control group showed a significant alteration in the diabetic direction. In other words, these lesions are closely connected with diabetes, as was earlier demonstrated concerning atrophic circumscribed skin lesions (Melin[42]).

Furthermore, erythema and necrosis were more common in patients with open diabetes compared to patients not known to have diabetes. The occurrence of these lesions is dependent upon the duration of diabetes. In younger patients they were only seen in those with a long duration of diabetes. In elderly patients with open diabetes and cutaneous erythema and necrosis, the duration of diabetes was significantly longer than in those with erythema alone.

The skin lesions described and precipitating factors An altered reaction in certain diabetics

Precipitating factors could generally be established for the skin lesions of the lower extremities described above, the most usual being cardiac decompensation with or without edema of the legs, but also deep venous thrombosis of the leg, nephropathy with edema of the legs or edema of the legs of unknown cause could be implicated. They may possibly be precipitated by peripheral arterial insufficiency For that reason these skin lesions are suggested to be due to an altered reaction in diabetics to certain precipitating factors. The precipitating factors were more common among patients not known to be diabetic than among those with open diabetes. This may mean that the assumed alteration in mode of reaction is less pronounced in patients of the former type, and that in these patients the skin lesions usually develop only in response to more powerful precipitating factors. As mentioned above, the connection in time between precipitating factors and these skin lesions of the lower extremities was obvious.

Entmacher et al. (21) state that cardiac disease is 2 0 times as frequent a cause of death among diabetic men as among men in general. The corresponding figure for women is given as 3.2. Of the precipitating factors mentioned above, cardiac decompensation would therefore be expected to be more common among diabetics than in the population at large. Whether leg edema of other cause than cardiac decompensation is more common among diabetics than in the general population is not known. In Sweden the frequencies of recognized diabetes in the age groups 60-69 and 70-79 are respectively 2.6 % and 4.3 % for men and 3.3 % and 4.2 % for women (49) The factors precipitating the above-mentioned skin lesions can be assumed to be 2.0 and 3.2 times more common among diabetic men and women, respectively than in the population at large Our finding that about 53 % of the men and about 76 % of the women with cutaneous erythema, with or without necrosis, had open diabetes, further implicates these skin lesions as being related to diabetes.

The lesions described were observed in patients with maturity-onset diabetes. They

were also observed in young diabetics but only in those with long duration of the disease. The elderly diabetics with erythema and necrosis had longer duration of diabetes compared to those with erythema alone. Erythema and necrosis must be regarded as a more powerful reaction than erythema alone. Thus, the altered reaction is connected to the duration of diabetes.

That the lesions described were seen predominantly in elderly people is probably due to the fact that precipitating factors such as cardiac decompensation are more common in the older age groups.

There is no evidence that occlusive, arterial lesions in the lower extremities are of of any decisive importance for the development of the lesions described (I V and VI) It is, however to be noted that the question of peripheral arterial insufficiency was estimated with regard to peripheral coldness and absence of pulses in the arteries of the lower extremities. Arterial angiography was performed only exceptionally The clinical significance of arterial occlusions demonstrable by angiography is, however not clear Widmer et al (65) in an investigation of 6 400 industrial workers, performed angiography in 83 persons and demonstrated 99 peripheral arterial occlusions in 75 of them. Two-thirds of these 75 persons were entirely free of symptoms in the lower extremities.

The connection between cutaneous erythema with or without necrosis localized to the feet and skeletal lesions in the feet of diabetics

As mentioned previously the diabetics with the skin lesions described above more often had destructions and demineralization of the bones of the feet compared to diabetic control patients without these skin lesions. Destruction or demineralization of the bones of the feet were more common in patients with erythema and necrosis on the feet than in those with erythema alone. The occurrence of factors known to precipitate the skin lesions of the feet was more common in patients with skeletal destructions than in patients without these destructions As mentioned above, the connection *in time between precipitating factors* and the development and disappearance of the skin lesions described was usually apparent (I) There are obvious reasons why the time of onset or the possible disappearance of the skeletal destructions could not be determined. The connection between the skin lesions and skeletal lesions, especially the fact that precipitating factors for the skin lesions were more common in patients with skeletal destructions than in those without, speaks in favour of the skeletal lesions as well as the skin lesions being precipitated by certain, well defined factors. It is plausible to suppose the skin necrosis and the skeletal destructions to be equivalent lesions localized to different tissues in the feet.

As mentioned above, there could not be demonstrated any significant differences in the occurrence of polyneuropathy between patients with both skin lesions and skeletal destructions and those with skin lesions alone. These findings weigh against the assumption that polyneuropathy alone causes skeletal destructions in the feet of diabetics (cf I)

Cutaneous erythema with or without necrosis localized to the feet, and distal gangrene

We compared patients with skin lesions of the feet in the form of distal gangrene to patients with cutaneous erythema and necrosis on the feet excluding distal gangrene (VI) No differences between the two groups of patients with respect to age duration of diabetes, occurrence of precipitating factors and the occurrence of skeletal destructions could be demonstrated. The

conception that there is no distinction between cutaneous erythema, with or without necrosis, and distal diabetic gangrene is supported by these latter results. The same factors are of importance for the development of the lesions.

Lesions of the legs in familial amyloidosis with polyneuropathy

The conformity between the described skin and skeletal lesions in these patients with amyloidosis and in diabetics is striking. Spontaneous blisters described in these patients correspond to bullosis diabeticorum (14 40, 57)

Altered reaction to local thermal trauma in diabetics patients with familial amyloidosis and polyneuropathy and alloxan diabetic rats respectively

As to clinical observations of certain skin lesions in diabetics, we have suggested that diabetics, or certain diabetics have an altered reaction to factors precipitating these skin lesions and that this altered reaction is connected with the duration of diabetes.

On comparison of the reaction to local thermal trauma on the skin of the extremities in diabetics and controls, differences were directly demonstrated. Purpura within the area of traumatization was observed more often and were more abundant in diabetics than in controls, especially on the lower extremities. This was true for young diabetics as well as for those with maturity onset diabetes. In young patients with diabetes the occurrence of purpura was *dependent upon the duration of the disease.* Purpura was, however also seen in a few patients with diabetes of short duration. Among the controls, purpura was observed only in the elderly and only on the legs. In addition to purpura, diabetics with atrophic circumscribed skin lesions on the lower extremities often also developed an intensely reddened border round the cutaneous area of traumatization. They eventually developed atrophic circumscribed skin lesions at the sites of traumatization. Almost all of these patients had juvenile diabetes of long duration or maturity-onset diabetes. This phenomenon was never seen in the controls. The findings corroborate the suggestion of an altered reaction and its relation to the duration of diabetes.

In an identical investigation concerning thermal traumatization of the skin on the extremities in patients with familial amyloidosis and polyneuropathy purpura was observed more often within the area of traumatization than it was in controls. Some of these patients developed atrophic circumscribed skin lesions at the site of traumatization.

Concerning alloxan diabetic rats there were also demonstrated differences in cutaneous reactions to local thermal trauma compared to control rats. The differences were related to the degree of cutaneous erythema within the area of traumatization. *The factor of long-term diabetes* was of decisive importance for the intensity of the dermal reaction. The diabetic metabolic derangement per se and the age of the animals were also of importance.

Explanation of the altered reaction in diabetics, patients with familial amyloidosis and alloxan diabetic rats respectively

In the following discussion which aims at explaining the demonstrated differences between diabetics and controls and between patients with amyloidosis and controls, *respectively* we should keep in mind the fact that capillary lesions, so-called diabetic microangiopathy is common in certain diabetics. Quantitative measurements of basement membrane thickness demonstrate that this is dependent upon the duration of diabetes (39 53 54). The basement membrane thickness is increased in the lower extremities as compared to the upper

extremities (1 62) The capillary basement membrane is normally thicker in the lower than in the upper extremities even in non-diabetics (66) the thickness increasing with age (39). In alloxan diabetic rats the age of the animals and the duration of diabetes, is of corresponding importance to the anatomy of the small blood vessels or corresponding tissue structures (28) Diabetics with microangiopathy often have peripheral polyneuropathy especially in the lower extremities (27 47) The presence of peripheral neuropathy in diabetics impairs the capacity to vary the blood circulation to the skin area in question (24 47)

Patients with familial amyloidosis and polyneuropathy also have an impaired capacity to vary the circulation through the distal parts of the lower extremities (3) They have not been observed to have signs of occlusive arterial disease (3 IV) There are amyloid deposits in the walls of the small blood vessels of these patients (3 32).

The presence of diabetic microangiopathy as well as peripheral neuropathy with impaired capacity to vary the blood circulation distally in the lower extremities will probably impede the passage of oxygen from the capillary blood to tissue cells as well as the passage of metabolites in the opposite direction. Other authors (1 10) have also suggested that diabetic microangiopathy could make diffusion through the walls of the capillaries more difficult. An impaired diffusion has not been demonstrated experimentally (67) but the experimental conditions were not satisfactory

The investigation of alloxan diabetic rats demonstrated that the diabetic metabolic derangement per se is of importance for the cutaneous reaction to traumatization. The altered reaction to traumatization observed in a few patients with juvenile diabetes of short duration might be due to the diabetic metabolic derangement per se or to the possible presence of an earlier latent diabetes with secondary microangiopathy The fact that an altered skin reaction to traumatization was not demonstrated, as a rule, in young patients with juvenile diabetes of short duration, is evidence that the diabetic metabolic derangement per se is not of great importance for these altered reactions in diabetics.

Precipitating factors such as cardiac decompensation may possibly release the tissue reactions by a decreased blood flow through the distal parts of the lower extremities. The presence of edema may impair the passage of oxygen and metabolites by interposition or by bringing about a compression of the small blood vessels.

The altered cutaneous reactions to local thermal trauma in patients with familial amyloidosis and polyneuropathy may be explained in the same way It is noteworthy that these patients were not diabetic.

A comparison of our description of certain skin lesions in diabetics with earlier descriptions in the literature

Diabetes is a common disease. The skin lesions described have a characteristic appearance and are common in diabetics. The question is then, if these lesions have been described earlier then by what names? In this connection, however it is noteworthy that an atrophic circumscribed skin lesion in the lower extremities of diabetics (Melin) was first described as late as in 1964 in spite of this lesion having a characteristic appearance and being found in about 65 % of male diabetics and in about 30 % of female diabetics (45)

As mentioned above, cutaneous erythema with necrosis is understood as a kind of diabetic gangrene and cutaneous erythema without necrosis as incipient gangrene. Diabetic gangrene is not only localized to

the acra of the lower extremities but can also be localized to the dorsa of the feet and sometimes appears as patchy areas of the lower legs. Information of erythema surrounding the areas of necrosis is lacking. As to the patchy areas of necrosis this may be due to amputation specimens being studied. Cutaneous erythema on the lower extremities has been mentioned by Ipsen. Attention has not been paid in the literature to this information and information about the frequency of erythema in connection with diabetic gangrene is lacking. Erythema or other skin lesions of the feet concomitantly with Charcot joints in diabetics have however been described (43 61). A pronounced and widespread, reddened border zone is, according to our experience, not only typical of diabetic gangrenes but also makes the appearance of them different from that of cutaneous gangrenes and distal gangrenes caused by occlusive arterial disease of the lower extremities in, for instance, patients with polyarteritis nodosa. In the latter group of patients we have never seen a reddened border zone corresponding to that found in diabetics. The phenomenon of reddened border zone is typical of spontaneous as well as of traumatic, recent efflorescences in atrophic circumscribed skin lesions in the lower extremities in diabetics.

Erythema of the skin in connection with arterial ischaemia has been described earlier (41). Ischaemic ulcers of the legs (19 20, 33) and so-called hypertensive ulcers of the legs (44) may on the basis of appearance alone sometimes be difficult to distinguish from the skin lesions described here — cutaneous erythema with or without necrosis. However among other things, ischaemic ulcers and so-called hypertensive ulcers are reported to be painful. The ulcers described by us are nearly always painless. Concerning the ischaemic and so-called hypertensive ulcers of the legs, they have been described without mention of whether diabetes was present or not.

Purpura and pigmentation localized to the lower extremities and yellow toe nails, to our knowledge, have not been described earlier in connection with diabetes.

Addenda

1) In diabetics, the skin lesions described and most probably also the skeletal destructions in the feet and distal gangrene, often or most often are precipitated by certain factors, mostly cardiac decompensation with or without edema of the legs and feet. For that reason it ought to be possible to influence the occurrence of the lesions described by adequate treatment of the cardiac decompensation and of the local edema. The lesions may be expected to heal faster after such a treatment. In our experience, this treatment is of decisive importance to the course of healing. Regression of cutaneous erythema, with or without necrosis, and of skeletal destructions have been described in earlier articles (I IV and VI) and also in this paper

2) It is well known that burns often cause severe skin lesions in the lower extremities of diabetics. This has been related to decreased sensibility caused by diabetic neuropathy (64). In diabetics, the altered cutaneous reaction of the lower legs and

feet must be of importance for the development of skin lesions caused by traumatization e.g., burns. The conditions might probably be the same for the patients with familial amyloidosis and polyneuropathy 3). In an earlier article (I) we summarized the clinically demonstrable lesions in the lower extremities of elderly diabetics. That summary should be revised on the basis of more recent findings. Purpura, pigmentation and yellow toe nails had not been described at that time. Bullosis diabeticorum (cf IV) was excluded, among other things, because of the rarity of this condition. However during a period of seven years we have observed and registered five patients with this lesion.

It is doubtful if arthropathia diabetica (so-called Charcot joints) secondary to peripheral neuropathy should be accepted as a particular clinical condition. In the literature skeletal destructions in the feet of diabetics on one side and arthropathia diabetica (so-called Charcot joints) on the other side are considered as equivalent lesions (e.g. 12, 43 61). This is not justified. Our study of patients with cutaneous erythema, with or without necrosis, on the feet demonstrated that many of these patients also had skeletal destructions in the feet. These skeletal destructions and skin lesions probably are equivalent lesions localized to different tissues in the feet. As far as we can judge, it is uncertain if the syndrome of arthropathia diabetica really exists, that is if it is defined as joint lesions corresponding to those in the knee joints and other joints in patients with tabes dorsalis and which are mainly caused by decreased sensibility. The difference in the appearance of the skeletal lesions in diabetics and in patients with tabes dorsalis has also previously been pointed out by Azerad (9).

In an earlier article (I) we made a distinction between cutaneous erythema, with or without necrosis, on the lower extremities in diabetics and distal diabetic gangrene. It is, however probably more correct to term cutaneous erythema with necrosis as diabetic gangrene and cutaneous erythema without necrosis as incipient diabetic gangrene. As mentioned above, diabetic gangrene localized to the lower extremities is always surrounded by a reddened border zone. Due to historical reasons, however we have chosed to use terms such as distal diabetic gangrene and cutaneous erythema, with or without necrosis. We have earlier reported (V) that redness of the toes is quite typical for older diabetics and is usually present concomitantly with rubeosis plantarum. Mal perforant plantaire is understood as diabetic gangrene with a particular localization and in which mechanical traumatization is of importance and where the sensibility is obviously decreased.

To sum up, the clinically demonstrable and for diabetes more or less characteristic lesions in the lower extremities are as follows: 1) Distal gangrene. In our experience characterized by a surrounding zone of erythema. 2) Areas of cutaneous erythema, with or without necrosis, localized to the legs and feet. Cutaneous erythema with necrosis is a kind of diabetic gangrene and cutaneous erythema without necrosis is incipient gangrene. 3) Rubeosis plantarum, including redness of the toes. 4) An atrophic circumscribed skin lesion (Melin) most often called dermopathia diabetica in the literature (cf I). The initial lesions are usually surrounded by a reddened border zone. 5) Bullosis diabeticorum. 6) Purpura and pigmentation. 7) Yellow toe nails. 8) Necrobiosis lipoidica diabeticorum. 9) Peripheral polyneuropathy. 10) Skeletal destructions or demineralization localized to the feet.

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Supplementum 590

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A Clinical Study Based on Patients Living in Northern Sweden

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Familial Amyloidosis with Polyneuropathy

A Clinical Study Based on Patients Living in Northern Sweden

BY

RUNE ANDERSSON

Umeå 1976

This account is based upon studies stated below It also contains informations based on the whole patient material which have not been published previously particularly concerning the development of the disease and about genetics

I ANDERSSON, R & KASSMAN T : Vitreous opacities in primary familial amyloidosis Acta Ophtal (Kbh) 46 441 1968

II ANDERSSON R : Hereditary amyloidosis with polyneuropathy Acta med scand 188:85 1970

III ANDERSSON R & BLOM S : Neurophysiological studies in hereditary amyloidosis with polyneuropathy Acta med scand 191:233 1972

IV ANDERSSON R & HOFER P -Å : Genitourinary disturbances in familial and sporadic cases of primary amyloidosis with polyneuropathy Acta med scand 195:49 1974

V ANDERSSON R & HOFER P -Å Primary amyloidosis with polyneuropathy Some aspects on the histopathological diagnosis ante mortem based on studies of biopsy specimens from 30 familial and on-familial cases Acta med scand 196:115 1974

VI ANDERSSON R & BJERLE P : Studies of urinary bladder dysfu ction in amyloidosis with polyneuropathy Acta med scand 197 117 1975

VII ANDERSSON R & BJERLE P Periph ral circulation particularly heat regulation reactions in patients with amyloidosis and polyneuropathy Acta med scand In press 1976

VIII HOFER P -Å & ANDERSSON R : Postmortem findings in primary familial amyloidosis with polyn uropathy Acta path microbiol scand Sect A 83 309 1975

These papers will be r ferred to in the text by th ir Roman umerals

C O N T E N T S

INTRODUCTION

A few isolated cases of primary amyloidosis with polyneuropathy had been described earlier (17 30 43) when several cases were reported in 1952 from Portugal (1) The disease was confined then as a clinical entity The familial occurrence of the disease was pointed out also Only a few reports of simila familial amyloidosis were to be found before from other countries (18 19) when some cases of this disease were diagnosed in 1965 at the Department of Internal Medicine University Hospital Umeå (I) Since then further cases have gradually been found in the north of Sweden Most of them have been familial Also cases so far considered sporadic have been diagnosed

One isolated case of primary amyloidosis where peripheral polyneuropathy was the characteristic manif station had been reported from Finland i 1954 (33) whil familial occurrence of amyloidosis with polyneuropathy had not been previously reported from the Nordic Countries (Denmark Finland Iceland Norway and Sweden) It was therefore considered justified to analyse more closely the character of the disease on the basis of the existi g material in northern Sweden This material comprised 60 patients of whom 42 were familial

The aim was in the first place to study the clinical manifestations of the disease and the possibilities of diagnosing the disease As the histologi al proof of the amyloid substance is of decisive importance for the diagnosis of amyloidosis and as the deposition of amyloid in various organs is probably of importance for the clinical manif stations particular attention was given to the histopathology of the disease The familial occurrenc was also studied Symptoms of the disease were not confined to the peripheral nerves but also con ected with many other organs Certain disturbances for example in connection with function of the urinary bladder peripheral circulation and to a certain extent also function of the gastrointestinal tract were studied in greater detail The polyneuropathy was studied with europhysiological methods Factors which affect the time of survival from onset of symptoms till death were watched Questions concerning the conformity of the cases under observation with those

which were described in Portugal and other countries were dealt with Statistical genetic calculation was performed on the material which had been collected

M A T E R I A L

The primary patients with the disease were discovered in the routine medical service at Umeå Hospital (I) Since the occurrence of this disease in northern Sweden had become more well-known further cases were diagnosed also in other hospitals These cases were kindly placed at disposal for this investigation Two families were studied more closely in connection with the appearance of the disease On the other hand there was no complete inventory made concerning the total number of people suffering from the disease in the region

The patient material for this account is listed in Table I It comprised 60 patients * The cases are designated in the table with clinical numerals 1 60 Genetic numerals referring to pedigrees Figure 1 - 15 are also given as well as designations from previous publications The cases were collected in accordance with the following three groups:

Group 1 Consecutive cases which were diagnosed in northern Sweden from Autumn 1965 to Summer 1974 They had all polyneuropathy Amyloidosis was confirmed by histopathological examination of biopsy material The majority of the cases were diagnosed at the Departments of Medicin in Umeå and Skellefteå Information of new cases was received also from the Department of Neurology Umeå and from hospitals i other parts of the region In this group there were 49 cases Sixte n of these cases had died by Summer 1974

Group 2 Six cas s of which 4 were relations to patients in group 1 They had died before this study started Clinical data were taken from hospital records Pathologico-anatomical diagnosis could be substantiated by re-examination of biopsy or autopsy material This group included cases with the clinical numerals 5 10 21 36 45 and 54

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* Since the completion of this investigation about 10 more cases have been diagnosed in the region

Group 3 Five cases deceased close relations (sibli gs or children) to patie ts in group 1 without pathologico-anatomical conf rmatio of amyloidosis According to hospital records however they had a typical clinical picture of advanced polyneuropathy In the cases with cli ical numerals 3 4 and 35 amyloidosis was histopathologically confirmed in their brother or sister In two cases no 12 and 13 there was a reliable report of se ious polyneuropathy in one of their respe tive par nts Neithe b opsy or autopsy had been carried out on thes patients The diagnosis is discussed under th heading GENETICS

Fortytwo of thes 60 patients belonged to 15 families whose pedigrees are described in Figure 1 - 15 Eighteen we e regarded as sporadic clinical numerals 43 - 60

In this account the result of genealogical studies especially regarding two families Figure 1 - 2 is also presented The material for these gen alogical studies was collected by information from the patients themselves from pastorates in various parish s and from the Provincial Archive in Härnösand as well as by informatio from two genealogists Mr Ossian Egerbladh Ph D and Mr Bertil Lindqvist Microfilmed copies of parish records available at th County Library in Umeå were studied personally by the author

I addition to the aforementioned 60 cas s 20 members of family 1 and 50 members of family 2 we the subject of cli ical examin tio by the author Som of the seventy relations exami ed had cli ical symptoms which agreed with those found i the amyloidosis patients Despit repeated biopsies no amyloid howev r could be detected in those cases They will be discu sed u der th heading GENETICS

M E T H O D S

P iph ral polyneuropathy

Diagnostic criteri of polyneuropathy wer partly anamnestic information partly findings at clinical exami ation

A. Anamnestic information concerni g dysesthesia paresthesia

and loss of sensibility as well as concerning progressive muscular wasting and weakness

B Findings at clinical examination pointing to sensory disturbances (tests by pin-prick cotton-wool hot and cold water in a testtube and tuning fork and test of the sense of position) and motor disturbances (muscular atrophy and reduction of strength) Examination concerning spontaneous muscular fasciculations as well as tendon reflexes in arms and legs was performed

These clinical examinations were supplemented in the majority of cases by neurophysiological examination Electromyography and estimation of the motor conduction velocity were carried out on 34 patients according to methods described previously (III)

A grading of the symptoms and signs from the peripheral erves was done clinically (III) + means slight ++ moderate and +++ adva ced degree of polyneuropathy It should be pointed out that the gradation used was only semi-quantitative It was used to give an approximate conception of the neuropathy at the time of the actual exami atio

Amyloidosis

Examination concerning the occurrence of amyloid substance in biopsy specimens and autopsy material was performed according to methods described previously (V VIII) Amyloidosis was considered confirmed if staini g with alkaline Congo red (32) showed deposits of a substance which in an ordi ary light microscope gave a bright green colour when vi wed in polari ed light (28)

Statistics

The statistical studies compr sed only calculations concerning median and mean values standard error of mean standard deviatio S D sta dard rror of standard deviation and mean differe ce The significance of the diff r ce between two means was calculated according to Fischers test

RESULTS

Sex a d Age Distribution

Of the 60 cases in the clinical material 40 were men and 20 women Table I These umbers give the proportion of men women as 2:1 Of the familial cases 27 were men and 15 women 1 8 1 The cases considered as sporadic consisted of 13 men and 5 women This gives the proportion of men to women as 2 6:1 in this group

In 49 cases the diagnosis was established ante mortem by examination of biopsy specimens Age at the time of examination when the diagnosis was established can be seen in Tables I and III The median age was 61 years and the mean age 59 8 ± 12 9 (S D) For 33 men the median age was 63 5 and th mean age 61 8 ± 13 6 Sixteen women had the median age of 59 5 and the mean ge of 55 5 ± 11 8

A comparison between familial and sporadic c ses showed that for the 33 familial cases the median age was 61 years and the mean was 60 1 ± 13 2 (S D) while the 16 poradic cases had median age of 58 5 and a mean age of 59 1 ± 12 7

In th group of familial case 21 proband had at diagnosis a median age of 66 years and a mean age of 63 9 ± 13 2 (S D) For 12 secondary cases in this group the median age was 56 and the mean age was 53 5 ± 10 8 Statistically the diffe enc between probands and econdary ca es i this respect was significant (0 05 $>$ p $>$ 0 01)

Age at Onset

The age at th time of the initial symptoms of the disease can be seen i Tables I and IV For the 60 patients as a whole the median age in thi respect wa 53 5 years and the mean age 53 0 ± 11 4 (S D) range 29 75 years For 40 men the median age was 55 5 and the mean ge 54 4 ± 11 9 The media age for 20 women was 51 5 and the mean age 50 1 ± 9 7 The difference between men and women was not sig- ificant (p $>$ 0 1)

When a comparison was made between the groups of familial

and sporodic cases no difference was found The median age for 42 familial cases was 54 years and the mean age was 52.6 $\pm$ 11 7 (S D) For the 18 sporadic cases the median age was 54 and the mean age was 53 9 $\pm$ 11 9

It must be pointed out that the times given regarding the onset of the disease in some cases were uncertain This was particularly so for the older patients who probably had had symptoms of the disease for several years The times were also uncertain with regard to what was defined as initial symptoms in a disease such as this with a widely varied pattern The symptoms which were interpreted in this study to be the earliest manifestations of the disease are given in Table I

It was thought feasible that the information might be somewhat more certain for the patients who were diagnosed ante mortem These 49 cases were analysed separately as regards age when the symptoms began The results are shown in Table IV No difference was discovered from the data already given

In compa ing probands and secondary cases within the group of familial cases there was no significant difference as regards age of o set

Neither was there any significant difference in age of onset between the patients with pronounced diarrhoea and those without that disturbance (see below regarding the development of the disease)

Pathology

The diagnosis of amyloidosis is in principle dependent on the histopathological ami ation and the evidence of amyloid deposition in various tiss s

A detailed investigatio of 4 autopsy cases was performed This material was supplemented by autopsy material from another two cases All i cas s were familial The result was reported separately (VIII)

It is of i terest to ote that in th se cases of amyloidosis there were no macroscopic organic changes which indicated the disease

Liver and spleen were not enlarged Their parenchyma had no wax-like or lardaceous character

When examined microscopically however amyloid deposition was found i various tissues An abundant and wide-spread deposition of amyloid was found in peripheral nerves Amyloid was also found in spinal ganglia and nerve roots There was also wide-spread depos tion of the amyloid substance in various parts of the autonomic nervous system Varying amounts of amyloid were discovered in the meninges On the other hand the central nervous system itself was essentially unaff cted Some atrophy for example in the anterior horns in the spinal cord was found in a few cases

Amyloid was also often found present in many other organs and tissues In our investigation there were deposits particularly i th walls of blood vessels The vessels affected were of various calibre

Amyloid was also wide-spread in conju ction with smooth muscles and perivascularly i the connective tissue

I the liver amyloid was observed only in the vessel walls of the portal triad Amyloid was found also in the spleen in very small quantiti s There it was found only in the walls of arteries of various size and not in the red pulp No amyloid was seen in the islet of Langerhans in the pancreas Neither was any significant amount of deposition found in the parenchyma of other endocrine organs In the kidney the affection was more varied Sometimes it was only in lesser quantities in the ma row In other cases there was ab ndant deposition even in the glomerules

Experience from autopsy material (VIII) and from biopsy examinations (V) formed the basis fo certain conclusions concerning suitable biopsy material for the diagnosis of this form of amyloidosis This will be discussed more closely u der the heading DISCUSSION

Clinical Manifestations

As was stated in the chapter on Pathology deposits of amyloid were fou d in this disease to be very widely spread in the peripheral erves and also in the autonomic nervous system This affection explains

the clinical pattern of progressive sensory-motor polyneuropathy and of more or less prominent manifestations indicating disturbances in the autonomic nervous system

Other localizations of amyloid deposits e g adjacent to smooth muscles in the walls of blood vessels and interstitially in varying amounts in the parenchyma of various organs show lesions which give conditions for clinical manifestations of different kinds Therefore in this disease there are grounds for a polymorphous and very varying symptomatology

After the report of initial symptoms found in the patients in this study special interest will be given in this chapter to the manifestations from certain organs

Initial symptoms

As was found in our first patients (II) and as was seen in all the material Table I the initial symptoms were usually sensory disturbances in the peripheral nerves They always began in the lower extremities Various forms of dysesthesia and paresthesia occurred Severe ache in the lower legs and brief attacks of shooting pain came initially in some cases Many patients reported an increased sensitiveness to cold as well as coldness in the feet as early and very troublesome phenomena

Symptoms of motility disturbances in the gastrointestinal tract appeared early in some of the patients This could occur even earlier than symptoms in the legs Also impotence sometimes appeared early Disturbance of vision due to vitreous opacities was reported as the first symptom in three patients

Periph ral rves

Sensory manifestations Initial symptoms usually consisted of various sensory disturbances Table I Various types of pain occurred Irregular attacks of sharp shooti g and burning pain were often the most troublesome There could also be a deep stabbing and more prolonged ache especially in the muscles of the calf Some patients complained of muscle pain in the lower legs induced by exercise (II VII) Paresthesia of varying intensity oft occurred such as prickling formication and burning

The impression is gained that these various sensory irritative phenomena were much more pronounced in this disease than usual in most other forms of chronic polyneuropathy e g in diabetes mellitus

Many patients reported that a pronounced sensitiveness to cold and cold feelings in the feet were early and troublesome phenomena Table I As part of polyneuropathy such phenomena are usually regarded as cold paresthesia Whe it concerns these patients with amyloidosis they were the subject of a special study (VII) The result is commented on in the following chapter about the cardiovascular system

In all patients there was eviden e of a reductio of se - sibility In clinical examin tion it was found th t the superfi ial sens of feeling was affected earlie and more notic ably than the deep It was also found that a certain dissociation occurred Thus the sensibility of superficial pain as well as the feeling for heat was ff cted on the whole more than that for light touch and cold Many pati ts stated that th y could ot feel th difference b tween h t and cold bath water with thei feet Th senses of vibration and of position were least affected It is of importance to be aware of this dissociation at the clinical examination of patients with suspected disease at an early stage

The pper extremities were affected late I these there was usu lly somewhat les intensity in the sensory irritative phenomena

Th sensory def cts had usually a typical symmetrical glove-and stocking distribution They progressed successively in proximal direction In advanced stag s there was a compl te loss of sensibility affecting all qualities

The red ced feeling for he t and pain was of importance for th origin of such complications as burns and s alds which could arise in connection with hot baths and attempts to heat the extremities locally

Motor manif stations The affe tion of the motor eurons probably occurs as e rly as that of the sensory Initially however they are not noticed in th same way by the patient Atrophy and weakness of the short toe extensors were found to be an early motor manifestation in thes patients (II III) Muscul r atrophy and

flaccid paralysis progressed symmetrically in proximal direction The gait became wide and stumbling A typical steppage gait with foot-drop developed In advanced stages total paralysis occurred distally in all limbs and pronounced weakness proximally also in the muscles of the trunk

At a later stage of the disease there were often fixed deformities in the distal joints of the extremities Among other things flexion-contraction in the finger joints was an example of this

It can be seen from Table I that abnormal muscular fasciculations appeared quite often This phenomenon occurred both in the muscles of the extremities and in the musculature of the to gue

The te don reflexes became weak successively It was found at clinical examination that particularly the ankle jerk was changed at an early stage The tendon reflexes of both upper and lower limbs were lost completely at a more advanced stage of the disease

Neurophysiological examination Electromyography (EMG) and estimatio of the motor conduction velocity (MCV) of peripheral nerves were performed in 34 patients Table I The findings confirmed th clinical diagnosis of polyneuropathy Th results of the neurophysiological examinations of several patients were accounted for s parately (III) EMG revealed fibrillations and denervatio potentials At volunt ry muscular contraction there were abnormal action potentials They could be polyphasic potentials with increas d amplitude and duration At maximal contraction a reduced number of moto units were activated

Th MCV was ofte pathologically prolonged It was observed however that MCV was less well correlated to the clinical pattern than EMG This can probably be explained by the fact that some efferent fibres with normal conduction velocity can be intact for quite a lo g time - Axonal degeneration is reported to be the predomina t form of myelinated fibre degeneration in this type of amyloidosis (11)

According to experiences of the investigation (III) MCV does not seem to reveal aberr tions as early as EMG does in this

form of amyloidosis The conclusion was formed that EMG in the musculature of the short toe extensors is a suitable form of examination in the early stage of the disease in order to confirm the clinical suspicion of neuropathy

In order to refine the diagnostics and to show the damage of efferent fibres the single fibre EMG (40) might be a more sensitive method Estimation of the sensory conduction velocity can probably also contribute to objectivising earli r the off c- tion of th nerves These methods however were not available at the time for this examination

In one patient with slight signs of polyneuropathy and with ve ified amyloidosis (no 16) the pattern was complicated by the patient having also progressive muscular dystrophy This disease occur ed he editary too i some members of her family The featur of myopathy predominated at the neurophysiological examination and the patient was classified as having myopathy

Autonomic nervous system Various manifestations which arose in these patients pointed to widespread affection of the autonomic ervous system as well From the clinical point of view however it was often not possible to differentiate the disturbances of these from those where local affection of other tissues e g blood vessels and smooth muscl s also occurred Various disturbances in which autonomic europathy was presumed to contribute to the manifestations observed are therefore discussed in connection with the respective organs

Histopathological exami atio Microscopical examination with regard to the nervous system revealed abundant and widespread amyloid deposition in th peripheral nerves (VIII) Amyloid was also found in the spinal ganglia and in the nerve roots Th re was widespread involvement of various parts of th autonomic ervous system as well On the othe hand th re was no d position in the ce tral rvous system itself

The abundant and widespread infiltration of amyloid substance in different part of the peripheral nervous system affects probably by direct local effect the function of the eurons It therefore contributes to the various clinical manifestations However as etiology and pathogenesy for the development of amyloidosis

nerves of the genital organs both in men and women (IV VIII) In men the affection of these nerves was interpreted to be the cause of impotence This disturbance was often present even in the early stage of the disease (II IV) To what extent the affection of the nerves of the female genital organs influenced their function was not examined in this study

Urinary bladder Disturbance of the function of the urinary bladder was found to be frequent It was analysed more closely (IV VI) To summarise it can be said that this disease often develops with the following disturbances or tendency to them:

1 Reduced bladder sensibility
2. Disappearance or decreasing of the contraction capacity of the detrusor musculature
3 Increased rigidity of the bladder wall
4 Increased bladder capacity
5 Overflow incontinence
6 Retention of urine with risk for urinary tract infection

Histopathologically amyloid was found in the vessel walls erves and smooth musculature of the wall of the urinary bladder (IV) It is probable that these various localisations of amyloid deposition cooperate to cause the disturbances that were found

Kidn ys Histopathological examination of 9 autopsy cases revealed very variable amounts of amyloid accumulation in the kidneys (IV) Deposits were fou d i the vessel walls in the cortex in 7 cases Four of these had also glomerular lesions which were pronou ced in two This varyi g affection of the kidn ys had variable clinical correlatio s too Thus increased val e of creatinine in serum was obs rved o ly i 5 of 26 patients e amined (IV) The creati ine was ormal i several patients with symptoms of the disease for ten years or more

P otei uria was fou d in 14 of 28 patients exami ed (IV) In 10 of them bacteriuri a d pyu ia wer also fo d Histopathologically there was the typical pi ture f chroni py lonephritis in 2 of the 9 cases exami ed

S rious kid y i suffic cy b caus of amyloid depositio a d/or pyelonephritis wa s ldom pr ent i these patients Only i

4 of 27 deceased patients was uremia a contributory cause of death Table II Nephrotic syndrome was confirmed in only one patient (IV)

Cardiovascular system

Both the heart and blood vessels of various calibres arteries and veins were found to be the seat of amyloid deposition as was the nerves of various parts of the cardiovascular system (VIII)

Heart Various manifestations indicating affection of the heart were stablished (II) It was conduction and rhythm disturbances that were most prominent Heart enlargement and failure were found more seldom

It is of course difficult to d termine to what extent other heart affection particularly cardiosclerosis contributed to the dysfunctions clinically observed as the patients mostly belonged to relatively high age group However when autopsy material was studied an amyloid deposition was often found to such a degree that the function of the myocardium might have been affected (VIII) Furth rmore amyloid was fou d in connection with the Purkinje fibr s This localization of amyloid might contribute to th occurrence of the conduction and rhythm disturbances that were observed

Complete A – V block was fou d in 3 patients Table I These patients r ceived treatment by a pace-maker

Blood pressure A low blood pressure was found i most patients Table I It should be noted that most of th se patients were of a relatively advanced age Blood pr ssure of 19 of the 50 patients exami ed was below 130/80 mm Hg A marked disposition to orthostatic hypotension was found in 10 of them Table I Most of these had no o only very slight increase of the pulse rate in connection with the fall of blood pressure when standing A disposition to syncope was found in some of them

The pathophysiological cause for low blood pressure and orthostatic hypotension was not analysed It seems to be likely however that the widespread amyloid involvement of the autonomic ervou system might be of importance

Peripheral circulation Investigation of peripheral circulation was performed The results were reported separately (VII)

As patients with amyloidosis and polyneuropathy often have signs and symptoms of circulatory disturbances in the extremities especially the legs such patients and controls were examined with oscillometry and digital pulse plethysmography in order to estimate the occurrence of possible arterial circulatory insufficiency No signs of significant obliterative arterial changes were found

Determination of skin temperature in fingers and toes during body-cooling and at subsequent indirect heating was also performed At low environmental temperature the skin temperature was higher in patients than in controls In some patients there was nearly no decrease of skin temperature despite a long period of cooling and a low rectal temperature At indirect heating a marked increase occurred in the skin temperature of the toes and fingers of the controls In most patients this reaction was completely absent in the toes The reaction was absent or reduced in the fingers of most patients as well These deviations can be explained by nerve damage caused by amyloid deposition in the nerves Amyloid deposits in the walls of small blood vessels may be an additional factor

It is suggested that the increased cold-sensitivity often experienced by the patients (see above concerning initial symptoms and sensory manifestations) is the result of the abnormal peripheral vascular response in the skin

Maximum blood flow in the anterior tibial muscle after combined ischemia and exercise investigated with radioactive xenon, was reduced in half of the patients examined (VII) Thus the blood supply of the skeletal musculature can be compromised too in these patients This is probably a contributory cause of the muscular discomfort experi nced by some patients during and after exertion (II)

Eyes

The occurrence of vitreous opacities was of great importance concerning th decision of diagnosis in our first patients (I)

In the material at hand vitreous opacities were revealed in 9 patients Table I No opacities occurred in 9 other patients The other 42 cases were ot adequately examined ophtalmologically in this respect

This affection of the vitreous body can b an early phenomenon of the dise se It was in fact reported as the initial symptom by 3 patients (no 2 3 and 50) Table I

The opacities in the vitreous body had a characteristic appearance with an amorphous white material in irregular glass-wool -like formations (I) They were often localized in th anterior part but also in the posterio part near the retina In advanced stages the opacities involved almost the whole vitreous body Vision was affected depending on the amount of deposition in varying degrees from floating dark patches and bands to total blind ess (I)

Anisocoria and irregular pupils with slow reflexes were also observed In 2 patients (no 7 and 54) typical Argyll-Robertson pupils with miosis were found Serological tests of luetic infection were negative in both cases

Histopathological exami ation of autopsy material revealed that the vitreous opacities had the characteristics of th amyloid substance (VIII) Amyloid infiltration was also observed in the vessel walls of the sclera chorioidea and retina Deposits were also found in vessel walls and in nerves around the globe of the eye On the other hand there was no amyloid found in the optic nerve itself

L rynx

Seve al patients had various degrees of hoa seness This was obvious in 20 cases Table I Seven patients had no hoarseness while it was difficult to judge in the rest of th c ses Seven of the patients with mark d hoarsen ss (o 2 6 7 9 10 11 and 24) were examined by an otorhi o-laryngologist No local changes of the vocal chords o of th larynx otherwis wer found Nor could any paresis or function distu ba ce of the vocal cho ds be discovered by routin examin tion Investigation with straboscopic light however was ot performed

Histopathological exami ation of autopsy material revealed amyloid deposits i the nerves as well as in th muscles of the vocal chord (VIII) These changes can expl in the disturbed function of the chords

It can be mentioned here that patient no 2 during her final year had difficulty with her breathing which had the character of stridor P tient no 6 had repeated attacks of difficult breathing

organs proteinuria was found in 14 out of the 28 cases examined (IV) Electrophoresis of the urine was performed in 4 cases (no 10 14 22 and 34) No Bence Jones protein was shown in these cases

Lumbar liquor Examination of the cerebrospinal liquor after lumbar puncture was carried out in 24 patients No pleocytosis was present in any case The content of protein was varying In one case (no 45) it was found 185 mg/100 ml As for the rest values between 16 and 83 mg/100 ml were found mean 50 mg/100 ml There was a concentration of over 50 mg/100 ml in 9 patients Electrophoresis on liquor protein was carried out in 16 cases No remarkable changes were observed

Diagnosis

Although various clinical manifestations especially in the case of familial occurrence very definitely can point to the actual disease histopathological verification is necessary to confirm the diagnosis of amyloidosis

In this type of amyloidosis skin and rectal biopsy were found to be valuable methods (V) It was found important that the biopsy material was representative In skin biopsy amyloid deposits were found especially in blood vessel walls in arrector pili muscles and adjacent to sweat glands These structures should therefore be included in the biopsy material Biopsy from the rectal mucosa must include the muscular layer to be adequate The submucosa with its blood vessels ought also to be included in the material

Biopsy of peripheral nerves e g the sural nerve was found to be a valuable alternative or complement (V)

At the examination of autopsy material no or only very little amyloid was found i the liver and spleen in several cases (VIII) These organs are th refore judged unsuitable for diagnostic biopsy examination of this disease

The degree of amyloid accumulation in the kidneys was found to be very varying Biopsy from this organ might be of value in certain cases

As amyloid deposition in the tissues sometimes can be only

mini al the h stopathological examination of biopsy specimens must be ca ried out thoroughly At routine exami ation stai ing should be done with alkali e Congo red and the exami ation performed in polarised light (V) W th ordinary light microscopy the amyloid appears faintly red I polarised light it shows the characteristic bright yellow-green olour

It can b difficult to rec ive at all times representative biopsy material This is perhaps particularly the case in the early stage of the disease In clinically suspected case repeated and complement ry biopsy specime s must be taken According to experiences from th i ve tigatio of th se patients in orthern Swede biopsies from the ski rectum d th sural rve can be recommended

As can be see from above this form of amyloidosis has a v ry varieg ted pattern It seems th refore to be of importance to diff rential diagnosis in many various situations not only i polyneurop thy The varyi g pattern of symptoms explai s the different in deq ate diagnose which had been given b forehand about the pati ts in this study Some of these diagnoses are given i Table II

Regardi g th five p tients i group 3 in this ccount (see Mate ial) th diagnosis of amyloidosis had not been established by histop thological evidenc The di gnosis in these cas s was based partly n the clinical manifestations partly on the h redit ry conditions These conditions will be discu sed unde the h ading Genetics

Dev lopment of the Disease

The time i terval from the i itial symptoms to the diagno is for the 49 cases which were diagnosed ante mortem can be seen in Table II Th i terval mentioned was 1 - 8 years median 5 years mean 6 $\pm$ 4 0 (S D) year There was o difference in this respect between men and women

Twentyseven of th 60 p tients have died Immediate causes of death s well as age at death have been given in Table II For all d ce sed patients the age at death was found to be as follows median ag 66 years mean age 65 6 $\pm$ 9 9 (S D) There was o significant difference between men and women

The duration from the initial symptoms till death is also given in Table II This information is summarised in Table VI For the whole group the median was found to be 9 years and the mean 10 7 ± 6 1 (S D) years For 20 men the median interval was 9 years mean 11 3 ± 6 9 Seven women had a median of 8 years and a mean of 8 7 ± 2 7 years There was no significant difference between men and women in this respect either

For the 27 patients who died 12 of them (no 9-14 21 22 33 34 43 and 47) had very pronounced gastrointestinal symptoms with diarrhoea early on in the development of the disease Tables I and II The age at death for these 12 patients is summarised in Table V Median age was found to be 61 and mean age 61 1 ± 7 4 (S D) As can be seen from Table VI the median for the interval between initial symptoms and death was 7 5 years and the mean was 7 3 ± 2 0 (S D) years

For the other deceased patients those who had no early pronounced diarrhoea 15 cases (Tables I and II) the information about age at death is summarised in Table V The median was 68 years and the mean age was 69 2 ± 10 3 (S D) years The time interval from the initial symptoms till death for these 15 patients was according to Table VI: median 12 years and mean 13 4 ± 7 0 years

The difference between these two groups of patients - those who had pronou ced diarrhoea and those who did not have this - as regards age of death mean age 61 1 ± 7 4 respectively 69 2 ± 10 3 is significant (0 05 > p > 0 01) As regards the time interval from th initial symptoms till death the difference - mean 7 3 ± 2 0 years e pecti ly 13 4 + 7 0 years - is also significant (0 05 > p > 0 01)

Incapacity for work

This disease showed gen rally a successively progressive development It was mostly th polyneuropathy but also th gastrointestinal disturba with diarrhoea a d malabsorption that resulted in incre s ng i valid ty and incapacity for work From Table II it can be seen that 32 of th se 60 patie ts 22 men and 10 women became complet ly able to work befor the age of 65 years

It can be s en too from Tabl II that many patients needed

cqnti uous care This was usually carried out in the home However besid s this it was necessary with repe ted periods of tr atment in hospital Some patients were cared for continuously in institutions for the chronic sick

Treatment

In amyloidosis secondary to chroni inflammation the development of the amyloid disease has been ob erved to be i fl nced favourably whe measures have been taken against the u derlying diseas (23 42) Otherwise ther is at the p ese t time o known efficacious treatment for amyloidos s One h s to turn to supportive and ymptomatic measu es

It is important to t eat supervening infections e g infection of th urin ry tract in th se patients

To patients with diarrhoea it i often difficult to give effe tive treatment against this disturbance Both malabsorption and malnutrition often arise This can be i fluenced to a certain xte t in diff rent ways Tre tment with a special diet consisting of ea ily digested food substan es has i some cases r sulted in a oticeable improveme t with weight inc ease and improved well-bei g (14)

The importance of adequate treatment of th skin le ions appea ing in the lowe limbs h s been emphasized (22)

Geographic Dist ibution

Birthplac s for the 42 familial and for the 18 sporadic cases are shown on th map Figure 16 Two distribution areas were found

1 Th i land re Twelve familial cases belonging to families 1 5 d 7 wer born in th inland Lappland The members of families 5 and 7 had most probably the same fo efathers as family 1 Th se forefathers were Fi ish immigrants who came to the ar a from the south-east i the 17th ce tury (12) This was also the case with the sporadi case in the inland area and with 2 of the

sporadic cases in the intermediary zone between the inland and the coastal area

2 The coastal area Most of the diagnosed cases were born in the coastal area and in the country nearest to the coast of the province of Västerbotten and Norrbotten

GENETICS

Background

The mode of inheritance in the Portuguese type of familial amyloidosis with polyneuropathy has been the subject of two investigations Becker et al computed the actual morbidity risk for the sibships of the patients to be about 40% (6) A drade et al analysed a numerically more important material distributed over 148 sibships (3) According to the maximum-likelihood-method a d assuming a complete selection they found the proportion affected per sibship to be 30 8% with a standard deviation of 2 3% Assuming a single selection the figures were 21 3% and 1 9% r spectively The results of the analysis of these two materials together with the pedigrees presented were considered to be in agreement with an autosomal dominant mode of inheritance

The patients who were included i Table I and who were the basis for the above ccou t had undoubtedly polyneuropathy Amyloid deposition was determined in 55 of the 60 cases As was stated before histopathological ami atio was not performed on the other 5 cases (see MATERIAL) Preliminary pedigrees of 2 families with some of these patients were reported p viously (II)

At an attempt to perform more detailed genetic analysis various difficulties arose depending on the ecessity of diagnostic certainty With a disease such as this it is ot always possible to mak a clear disti ction betwee a sick and a healthy i dividual I connection with the exami atio of relatio s of the affected patients there occurred som times anamn tically light symptoms which could be an expression of i cipie t ill ess but which could not be verified at clinical exami ation It could ot be exclud d that the disease in

some c ses had a very slow progression with only slight symptoms Sometimes the affection might have remained subclinical Thus to ascertain the diagnosis in the early stages was not always possibl in a disease such as this with an often i sidious development and with such a varyi g symptomatology The technical aids which were at hand e g for examination co cerning neuropathy (electromyography and estimation of motor conduction velocity) did not always seem to giv a sufficiently decisive result for a early diagnosis

Another difficulty in diagnosing this disease concerned the problem of revealing amyloid deposits in cli ically suspected cases by histopathological examination It is probably so that small biopsy specimens are not always enough to reveal amyloid The qu stion if th disease can develop without th appearance of amyloid depositions demonstrabl by the histopathological techn qu s used is discussed further on

No biochemical abnormalities of diag ostic s gn fica e e g in the blood or urine were known at the time of this i vestigation

Accordi g to th a count of material it was established that amyloidosis was histopathologically confirmed for group 1 and 2 Regarding group 3 clinical manifestations occurred i agreement with those cases which we e verified histopathologically The five pati ts in group 3 howev r were deceased and th re was no tissue material available for histological exami ation Gen alogically they b longed to families 1 2 or 10 and they wer close relations (brothers sisters or parents) to patients in group 1 The diagnosis was the efore based booth on li ical and ge e logical conditions

It is emarkable th t i families 1 and 2 several p tients were obs rv d to have distin t cli ical ma if stations as seen in familial amyloidosi with polyneur pathy but in whom amyloid deposits wer not found in biopsy examination Thes patients a e occounted for i the pedigrees Figure 1 and 2 and in Table VII and VIII The polyneuropathy in the extremitie wa graded in three degrees: + ++ and +++ (III) From Table VIII it can be seen that regarding the 49 cases where amyloid deposition was shown by biopsy xami ation the europathy in the legs i 8 ases belonged to g ade + i 15 to grade ++ and in 26 cases to grade +++ Thus it was ften possible to prove amyloid in those patients classified in

grade + and ++ Of those patients who are accounted for in Table VII no less than 6 had polyneuropathy in the lower extremities of grade ++ and grade +++ Despite this amyloid deposits could not be shown in examination of biopsy specimens

Thus it seemed that even in patients with marked signs and symptoms it was not always possible to establish amyloidosis by biopsy examination It was remarkable that it was not possible to prove amyloid depositions in autopsy material from patient 1:IX: 26 Table VII and Figure 1 Particularly the latter circumstance brought into question how much the clinical manifestations in these patients really depend on the amyloid deposits which could be shown by the histopathological technique used

Genealogical Studies

Pedigree 1 (Figure 1)

Several patients living or born in Lappland could be connected to one big family The pedigree over the family is shown in Figure 1 Most of the patients in that family belonged to different sibships in one and the same generation (generation IX) All their respective parents were deceased before this examination There was no information that any parent had manifestations of the disease However there was a dependable r port that the subject VII:11 became both lame and blind

It was established that the family had its origin from Finnish immigrant who came as colonists to Lappland in the 17th century (12) The earli st known forefathers in generation I lived in a very confined area i th south-ea tern part of Lappland They were most probably related to a h other (13)

As stated abo it was of great interest to note that further cases of europathy which was unmistakable clinically and neurophysiologically b t without demonstrable amyloidosis were diagnosed in this family Th s fact wa pr limi arily announced previously (II) The cases are pr ented i Table VII and VIII They are also taken up in the pedigre of this family Figur 1

Pedigree 2 (Figure 2)

The proband in this family was case no 9 (IV 74 B:1 in earlier publications) I formation was received that a brother had similar symptoms and that a deceased cousin had had polyneuropathy Several members of the family were examined A pedigree was drawn up (Figure 2) This family was traced back ge ealogically partly as far as to the 16th century No certain connection to family 1 could be proved N ither could any con ection to Finnish immigrants be reliably established

In this family too there were patients with cli ical manifestations such as are found in familial amyloidosis with polyneuropathy but in whom no amyloid deposits could be seen at biopsy examinations They are accounted for in Table VII and VIII and are shown in th pedigree Figure 2. When clinically exami ed most of these c s had only slight manif stations which could represent an early stage of neuropathy Two of them (case IV:38 and IV:47) had however clear and undoubtable polyneuropathy

Pedigree 3 - 15 (Figure 3 - 15)

In 24 f rther cases (clinical no 19 - 42) there was proved or probable familial occurren e of the disease These patients belonged to 13 smaller families (Figure 3 - 15) Some of them were traced several generation back genealogically N d finite connection with families 1 and 2 could be shown It an be noted however that birthplaces for many of these patie ts or th ir close forefathers were from a geographical point of view in the same are as those of family 1 or family 2

Sporadic cases

Eighteen cases were r ga ded as sporadic According to information from the patients themselves no similar symptoms of the disease existed in th i parents or in their brothers sisters or children Examination of their elatives however was not performed

Clinically and histopathologically no difference could be shown compared with the familial cases accounted for above It was not possible to decid whether these cases were genetically of the same nature as the familial

Analysis of Genealogical Observations

The data compiled by the genealogical studies were analysed statistically Taking the diagnostic problems into consideration which were accounted for above calculations were carried out concerning the proportion affected in sibships on the basis of material from pedigrees 1 - 8 and 10 - 14 (see Figure 1 - 8 and 10 - 14 and Table X) Because of i complete information concerning sibships in families 9 a d 15 (Figure 9 a d 15) these were not included in the calculations

The following p rsons were regarded as being affected:

1 Patients examined and shown to have definite and unmistakable polyneuropathy (solid symbols)
2 Patients who at the clinical examination had symptoms and/or signs indicative of polyneuropathy (half-filled symbols)
3 Patients who were reliably reported by their relations to have had unmistakable signs and symptoms of polyneuropathy (symbols with lines)

Those sibships were included in the statistical calculations in which at least one sib was affected and at least one had been exami ed Also those cases were included i the calculations where histopathological exami ation of biopsy and/or autopsy material had been u able to prove amyloid deposits

The description normal sibs was based partly on the result of clinical examination partly on information from other sibs Also sibs who had died before this study was performed were included Consideri g the results accounted for above concerning age at onset of symptoms those sibs were not i cluded who had died before reachi g the age of 25

The composition of the m terial can be seen from the pedigrees Figure 1 - 8 and 10 - 14 as well as Table IX - X

The proportio of affected sibs within the sibships was estimated according to the maximum-lik lihood-method (31) The calculation was performed 1) assumi g complete selection (complete ascertainme t) and 2) assumi g si gle selection (single ascertainment) - The selection is said to be complet when each affected individual is discovered because he or she is affected and not because of the discovery of the abnormality in a sibling (31) -

Assuming complete selection the proportion affected was found to be 26 1% with a standard deviation of ± 3 37 Assuming a single selection the corresponding figures were 17 9% ± 2 97 Considering the mode of procedure for gathering this mat rial the value of complete selection should be the most appropriate

The figures obtained were somewhat lower than those given by previous authors with regard to the Portuguese type of amyloidosis with polyneuropathy (3 6) The difference might depend on the higher age at onset of illness among the Swedish patients With regard to the pos ibility of reduced penetrance and higher age at onset the figures are consistent with the assumption of autosomal dominant mode of inheritance

The figures per se do not exclude the possibility of recessive inheritance This alternative however must be rejected although particularly pedigree 1 shows a high frequence of consanquineous marriages In all cases namely where parents could be examined one of the parents had the defect Furthermore the proportion affected sibs was not higher in th 15 families which had an affected parent than in the 26 families where the dis as did not occur in either of the parents (The 1 st named parents were not examined According to information given by their children no manifestations of the disease had appeared) Approximat calculation according to Li and Mantel (cf 20) gives the figures 26 1% and 25 9% respectively for these two groups The concist ncy of these figures agrees with dominant mode of inheritance With a r cessive mode of inheritance the proportion affected within sibships would be gr ater if one or other of the parents had the disease than if neither of the parents had the disease (50% and 25% r spectively)

The results of the analysis of family data together with the prese ted pedigrees are thus concistent with the assumptio of an autosomal dominant mod of inheritance Although the material for analysis was i completely examined th results indicate a reduced penetrance As it was stated above th age of onset of the disease was very varying From the surv y concerning the clinical material it was evident that there existed very variable expressivity in various respects Different kinds and i tensity of the manifestations as well as very varying development of the disease are examples of the variable expressivity

The expressivity was found to be not only individually different Also interfamilial differences were shown Thus the affection of the digestive system with troublesome diarrhoea was seen to be more predominant in certain families (family 2 4 and 10) than in other families This condition was of importance in the development of the illness This was more serious for the patients in family 2 than for those in family 1 The time interval from the beginning of symptoms till death can be seen on Table II For six deceased patients in family 2 it was 8 2 $\pm$ 1 8 (S D) years For the six deceased patients in family 1 the interval was 19 5 $\pm$ 6 7 years The difference is statistically significant (0 01 $>$ p $>$ 0 001)

The variable expressivity of the disease has not been particularly emphasized before As far as is known interfamilial differences concerning clinical manifestations have not been reported previously

D I S C U S S I O N

Various heredofamilial syndromes of amyloidosis have been described duri g the last years (cf 10 24) In some of them polyneuropathy is a always occurring manifestation more or less pronounced This has been the eason why it has been confined as a particular clinical entity The familial amyloidosis with polyneuropathy has been in its turn divided i to three types (4)

1 The Portuguese type (1 36)

2 The I diana-Maryland type (24 35);

3 The Iowa type (41)

The characteristic i type 1 and 3 is that the neuropathy begins in th legs Type 2 has neuropathic manifestations mainly in the upper e tremities most often as a carpal-tunnel-syndrome Type 3 is reported to have a high frequency of ephropathy with uremia as well as peptic lcer

A different type of hereditary amyloidosis with nervous involvement has been reported from Finland (26) Most characteristic for this disease is lattice dystrophy of the cornea and paralysis of the facial erves

In all of the patients diagnosed in northern Sweden the symptoms were first and most pronounced in the lower extremities It was therefore natural to compar the results of this investigation first and foremost with type 1 and to a certain extent also with type 3

Clinical aspects

The results of the examinations of the patients in northern Sweden showed in many respects agreement with what was reported earlier concerning type 1 especially from Portugal (1 2 5 8 9 29 and 34) Certain differences however were found

One difference to earlier reports concerning type 1 was that the age of onset in the Swedish patients was higher The mean age for onset in the existing material was around 55 From Portugal it was reported that the onset usually occurred between 25 and 35 years of age (4)

Steatorrhoea and other signs of malabsorption were rather common (II) Steatorrhoea was previously not reported from other countries Wh ther this difference is real can however not be proved

It is remarkable that peptic ulcer and gastroduodenitis occurred to such a wide extent among the Swedish cases The impression was gained that th same was valid for their relations too particularly in family 2 This fact shows a certain similarity to the cases which were reported from Iowa type 3 (41) On the other hand th cases seemed more compatible with the Portuguese as regards kidney affection (5) Serious kidney insufficiency with uremia as found i type 3 occurred only seldom

In this investigation of patients in northern Sweden studies were performed more closely of some clinical manifestations of the disease which were not or only partly taken into consideration by earlier authors They concerned malabsorption peripheral polyneuropathy dysf ction of the urinary bladder and signs indicating disturbance of the peripheral circulation distally in the extremities The r sult have been discussed already

It can be added here that chronic infection of the urinary tract has been regarded as being of etiological importance for the arisal of systemic amyloidosis (7) The results of the studies on the Swedish patients showed that amyloidosis with polyneuropathy was accompanied by dysfunction of the urinary bladder This disturbance brought the risk for infection of the urinary tract Thus in this form of amyloidosis infection of the urinary tract is probably a secondary consequence of polyneuropathy and not an etiological factor for amyloidosis

Histopathological aspects

As regards the histopathological findings the observations made in this material agreed in most respects with those reported from Portugal (36 38 and 39) It can however be noted that no significant amyloid deposition was found in these cases in the area of Auerbach s plexus in the intestinal wall Such a deposition has been stated to exist in a marked degree in the Portuguese cases (37) Whether this difference is real must however be left as an open question

With regard to the diagnostic methods for proving the presence of amyloid infiltration skin biopsy was considered to be a valuable method This is also in agreement with the Portuguese findings (39)

Rectal biopsy is regarded as being a suitable method to diagnose systemic amyloidosis (16 27) This method has not been more closely discussed earlier in this form of amyloidosis The result of e aminations of the prese t material showed that rectal biopsy was a valuable alternative or complement in this disease too

Pare chymatous organs were regarded as the most suitable for diagnostic biopsy procedure in type 3 (41) This is in contrast to the experience with patients in northern Sweden Only minimal amyloid deposits were found in the liver In many autopsy cases only very slight or no deposition at all was found i the spleen These findings are in agreement with those from Portugal (36) They show that biopsy from the liver or spleen is not to be recommended in diagnosis

Etiological aspects

According to the statements given above genetic factor is of etiological importance The possibility of other etiological factors however should be taken into consideration Although the clinical pattern and the histopathological changes are mainly the same some interfamilial differences as well as the appearance of sporadic cases may indicate heterogeneocity The possible importance of environmental factors on the development of this form of amyloidosis is quite unknown

S U M M A R Y

Sixty patients with amyloido is and polyneuropathy were studied The patients were born and lived in the north of Sweden In 55 patients the diagnosis was confirmed by the finding of amyloid deposits In 42 patients the disease was familial Eighteen cas s were classified as sporadic The disease was obs rved almost twice as much in men as in women

Th clinical manifestations of the disease as well as th histopathological changes were to a great extent in agr ement with those pr viously des ribed from Portugal Familial amyloidosis with polyeuropathy had not been described previously from the Nordic Countries (Denmark Fi land Iceland Norway and Sweden)

The disease is a ystemic disease Histopathological investigation of autopsy material showed that characterist c f ndings were amyloid deposits in the peripheral nerves including the autonomic in spin l ganglia and nerv roots in th walls of blood vessels of varying calibre i p ivascular con ective tissue and adjacent to the smooth mus les Liver and spleen wer involved to a very slight extent The deg ee of kidn y involvement wa varying Biopsy material from skin rectum and peripheral erve was found to be suitable for histopathological proof of amyloid deposition

Th patients had manif stations of the disease from several diff rent organs Polyneuropathy which began with various sensory disturbances distally in the lower limbs was a constant finding *Trouble-*

some irregular attacks of sharp shooting and burning pain often occurred A dissociated sensory loss was found with early impairment of pain and thermal sensibilities An early sign of the polyneuropathy was atrophy of the short toe extensor muscles

Accou ts were made about various clinical manifestations Attention was paid to the initial symptoms as well The age of onset varied between 29 and 75 years The mean age was 53 which was more than 10 years older than that reported from Portugal The age of onset was the same in men and women

The time interval between the initial symptoms and diagnosis was about 5 years Twentyseven of the 60 patients have died The duratio of the ill ess varied between 4 and 31 years and was in mean 12 years The majority of the 60 patients were incapable of work before the age of 60 - 65

The most predominant cli ical manifestations in addition to the europathy localised to the extremities were impotence disturbances of urinary bladder emptying constipation which was later in the development of the disease often followed by diarrhoea loss of weight visual disturbance in connection with vitreous opacities hoarseness low blood pressure and orthostatic hypotension cardiac rhythm disturbances as well as symptoms i dicati g circulatory disturba ces distally in the limbs

I dividual variations conc rning various ma ifestations of the dis ase were obvious I terfamilial differences regardi g cli ical manifestations e g gastrointesti al disturbance with diarrhoea occurred also The time of survival after onset of the disease was shorter in families where th diarrhoea was marked Steatorrhoea and oth r signs of malabsorption were often appare t in those patients Certai other manifestations of the disease were also st died more clos ly especially the peripheral polyneuropathy the disturbed function of th uri ary bladder and th symptoms indicati g circulatory disturba ces distally in the limbs

Neurophysiological studi s conc rning the polyneuropathy in the extremiti s sh wed that electromyography (EMG) as a diagnostic aid was superior to d termi ation of th motor conduction velocity of peripheral n s EMG e g of the short toe extensor muscles was found to be a val abl diagnostic method i the early stage of the disease

The disturbance of the urinary bladder function was characterised by reduced sensibility of the bladder disappearance or reduction of the contraction power of the detrusor musculature often an increased rigidity of the bladder wall increased bladder capacity overflow incontinence and urine retention with increased risk for infection of the urinary tract

In clinical physiological studies concerning the peripheral circulation in the extremities there was shown no sign of obliterative processes localised to the arteries of the extremities Arterial angiography which was performed in a limited number of cases did not show either any signs of artery obliteration At body-cooling and subsequent indirect heating it was found however disturbances of the vasomotor responses in the skin The ability of reactive hyperemia in skeletal musculature could be impaired too

The disease could develop with marked clinical symptoms e g polyneuropathy without it being possible to prove with available m thods any deposition of amyloid in the tissues where such usually appears in these patients

The predisposition of the disease is in all probability in herited and the mode of inheritance autosomal dominant with incomplete penetrance That this is the case was supported by the pedigrees presented and by the analysis of the proportion affected sibs within sibships

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A P P E N D I X

SURVEY OF CLINICAL DATA

A survey of some clinical data of the 60 cases of amyloidosis with polyneuropathy is given in Table I and II

Comments to Table I

Column 1 Clinical numeral Indicates identification number of each case reported in the clinical part of the study Cases designated with numbers 1 - 42 are familial ones while cases 43 - 60 are considered as sporadic

Column 2 Genetical numeral" The familial cases are designated by numerals referring to Pedigree-Generation-Individual in accordance with the pedigrees in Figure 1 - 15 In order to make possible the identification the designations that were used in previous reports (I VIII) are also presented here

Column 3 Sex M = male; F = female

Column 4 Age at onset of symptoms is given in years according to information given at the time of the examination noted in column 6

Column 5 Initial symptoms" The following abbreviations are used C cold ss of feet; H = hypalgesia and hypesthesia; P = pain in the lower limbs G = gastrointestinal disturbances O = opacities of the vitreous body I impotence; Ca cardiac symptoms

Column 6 Age at examination is given in years

Column 7 and 8 Peripheral neuropathy" of arms and legs is graded semiquantitatively as described previously (III) + = slight ++ = moderate and +++ marked neuropathy

Column 9 EMG and MCV" Electromyography (EMG) and motor conduction velocity (MCV) of peripheral nerves confirming the diagnosis of polyneuropathy is indicated by + not confirming by -

Column 10 "Fasciculations Spontaneous fasciculations appearing in the musculature of the limbs (L) and tongue (T) are noted 0" means that no fasciculations were observed at the time of the examination

Column 11 "Diarrhoea Daily frequent and troublesome diarrhoea at the time of the examination is indicated by +" No or only sporadic tendency to diarrhoea is indicated by 0"

Column 12. Marked weight loss occurring in 1 - 3 years is given by figures (kg) or by +" (approximately > 5 - 10 kg)

Column 13 Hoarseness The sign + indicates obvious hoarseness Normal voice at general talk is indicated by 0"

Column 14 "Vitreous opacities Examination by ophthalmologist: "+" typical opacities of the vitreous body without any other explanation than amyloid infiltration 0" = no opacities at the time of the examination

Column 15 "Blood pressure The value of blood pressure in mm Hg at recumbent and upright position respectively

Column 16 "Tissue specimens confirming amyloidosis The following abbreviations of biopsy specimens are used: N = sural nerve; S = skin; R = rectal mucosa; G = gastric mucosa; Gi = gingiva M = skeletal muscle

Column 17 Comments Some clinical manifestations of interest are given here Figures refer to the patients age

Columns 9 - 16 No sign is given in the table when examination was not performed or incompletely recorded or when information was uncertain

Comments to Table II

Column 1 "Clinical numeral See Table I

Column 2 Interval onset - diagnosis is given in years Onset refer to data in Table I column 4 Diagnosis refer to age at examination when biopsy revealed amyloidosis ante mortem The sign p m indicates that amyloidosis was proved post mortem In 5 cases histopathological examination was not performed: -

Column 3 Age at death in years

Column 4 Interval onset - death is given in years and refers to data in Table I column 4 and Table II column 3 respectively

Column 5 Immediate cause of death Information obtained from hospital records It refers to clinical statement

Column 6 Age at inability to work The figures indicate age in years when inability to work caused invalid pension Information from the patients their relatives or hospital records No sign indicate capability of performing usual work ? = no or uncertain information

Column 7 and 8 Age at need of nursing at home or at hospital Figures indicate age in years when physical handicap neces itated nursing help in daily life e g of dressing eating personal hygiene moving in bed getting into a chair

Column 9 Comments Some example of previous diagno s are given here as well as some examinations performed at ho pital

Column 3 - 5 No sign indicates that th patient was alive in summer 1974

Tabl 1. Survey of clinical data of 40 patient with amyloidosi and polyneuropathy

CLINICAL NUMERAL	GENETICAL NUMERAL: Pedigree	Generation	Individual	Earlier designation	Sex	Age at onset of symptoms	Initial symptoms	Age	Neuropathy: Arms	Neuropathy: Legs	EMG and MCV	Fasciculation	Diarrhoea	Weight loss	Hoarseness	I[?]	Blood pressure: Recumbent	Blood pressure: Upright	Tissue specimens positive for amyloid	COMMENTS
1	2				3	4	5	6	7	8	9	10	11	12	13	14	15		16	17
1	1	IX	47	A 1		50	CHP	44	+++	+++		LT	0			0	160/80		N S R GI	Duodenal lce 42
2	1	IX	21	A 2	F	53	O	63	+++	+++		LT	0	10			150/95	100/50	S	Duodenal lcer 33
3	1	IX	14	A 3		40	O	50	++	+++										
4	1	IX	30	A		35	CH	48	++	+++			0							Chronl lce l ft foot
5	1	VIII	18	5		44	HP	70	++	++			0	15					U inary bladde	Papilloma f inary bladde
6	1	IX	2	6		56	CHP	61	++	+++		LT	0			0	110/80	85/60	S R	
7	1	IX	8 a(II) A 7(IV V VIII)		M	50	PHD	46	+++	+++		L							S M	A gyll Robe tson pupil
8	1	IX	44		M	60	HP	64	++	+++		L	0						S R O	A-V block III Stok s-Adams ttack Pacemak
9	2	IV	74	B 1	F	56	HP	60	++	+++				23			110/80		S R	
10	2	IV	27	B 2	F	63	CHP	66	++	+++		LT		10			1 5/80		Spinal cord	
11	2	V	39	B 3	M	40	I	43	++	+++		LT		12			125/80		S R	
12	2	IV	23	B 4	M	57	H	60	++	++							135/90			Ga t i ention vomiting
13	2	IV	7	B 5	F	57	HD	64	++	+++										
14	2	IV	75	B 6	M	51	CH	57	++	+++		LT		18		0	105/70	75/	S	
15	2	V	43	B 7	F	33	HP	35	0	+		0	0	4	0		130/85		S	Ca duodeni l
16	2	V	114	B 8	F	40	H	43	0			0	0	0	0		140/90		S R	Hereditary muscular dy rophy
17	2	IV	77	B 9	M	60	CP	65		++		LT	0						S	
18	2	IV	79	B 10	F	53	CP	55	0			0	0	0	0		170/100		S	
19	3	II	1	C 1	M	56	HP	63	++	+++			0				140/90		Kidney	Chronl lce both fee
20	3	III	10	C 2	M	29	CHP	34	++	+++		LT		20		0	110/75		S R	
21	4	III	11	D 1	M	64	CP	67	++	+++							160/80	70/40	R	Ga itis. Orthostatl hypotension ack of syncope
22	4	III	10	D 2	M	63	PO	68	+++	+++		L					150/100	100/70	R	Hematoma l and melena 2
23	4	III	13	D 3	F	47	HP	60	++	+++		L	0	5			110/75		S R	Duodenal l 42 Charcot join (knee) Bullou dermatl
24	5	IV	23 a(II) E 1(III-V VIII)		F	54	HCe	63	++	++			0	7			175/100		R	A-V block III 3 wk s-Adams attack Pacemaker

[illegible]	[illegible]	130/80		[illegible]	
[illegible]	[illegible]	150/90		[illegible]	[illegible]
[illegible]	[illegible]	140/95		[illegible]	[illegible]
[illegible]	[illegible]	135/90		[illegible]	
[illegible]	[illegible]	130/90		[illegible]	
[illegible]	[illegible]	130/80		[illegible]	
[illegible]	[illegible]			[illegible]	
[illegible]	[illegible]	140/80		[illegible]	[illegible]
[illegible]	[illegible]	140/111			[illegible]
[illegible]	[illegible]	140/110		[illegible]	[illegible]
[illegible]	[illegible]	170/90		[illegible]	[illegible]
[illegible]	[illegible]	90/90			
[illegible]	[illegible]			[illegible]	[illegible]
[illegible]	[illegible]	160/90			[illegible]
[illegible]	[illegible]	170/90			[illegible]
[illegible]	[illegible]	190/90			
[illegible]	[illegible]	80/70			
[illegible]	[illegible]	170	90/60	[illegible]	[illegible]
[illegible]	[illegible]	130/90	90/60	[illegible]	
[illegible]	[illegible]	135/90	90/50	[illegible]	[illegible]
[illegible]	[illegible]	115/75	110/75	[illegible]	[illegible]
[illegible]	[illegible]	125/90	110/85	[illegible]	
[illegible]	[illegible]	110/90	85/60	[illegible]	[illegible]
[illegible]	[illegible]	150/90		[illegible]	
[illegible]	[illegible]	190/120		[illegible]	At [illegible]
[illegible]	[illegible]	130/80		[illegible]	
[illegible]	[illegible]				Gastroduodenal [illegible]
[illegible]	[illegible]	120/80	90/65	[illegible]	
[illegible]	[illegible]	150/80		[illegible]	
[illegible]	[illegible]	150/90		[illegible]	Bullae [illegible]
[illegible]	[illegible]	150/85	120/75	[illegible]	
[illegible]	[illegible]	[illegible]/95			
[illegible]	[illegible]	[illegible]	150/75	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]/75		[illegible]	
[illegible]	[illegible]	[illegible]/80			
[illegible]	[illegible]	[illegible]		[illegible]	

Tabl II Survey f l ical da (con nued)

CLINICAL NUMERAL	Interval onset of symptoms	Age at death	Interval onset death	Immed te cause of death	Age at nab / work	Age at home	Age at ?	COMMENTS Previous diagnoses ...
1	2	3		5		7	8	9
	1	70	70	Bronchopneumoni	62	64		
	0	48	12	Circul ry suffi iency	58		63	
3		5	1	Bronchopneumoni	47	4		My lography
4		57	72	Bronchopneumon a. Cachexi				Amyo rophi l l scl ro is. P ogre ive musculas dy t ophy
5	p.m.	77	31	C rculat ry i su ienc	7			P og ive muscul dy rophy
6	5				55	60		Subc rani polyneuropathy
7	15	68	18	Ci cul tory i su ency	60			
8	6							
9	4	61	9	Pulmonary embolism Bronchopneumoni	59	60	60	Ca myelog aphy
10	m.	71	8	Bronchopneumoni	65	68	69	Amy ophi l t ral scl rosi Malabsorption ith polyneuropathy Cli ical ?ase ? ?yl ide ?
11	3	46	5	Circul tory i suffi iency	43	5		Malabso ion ith polyneu opathy
12		65	6	?	58	59		
13		67	10	Cerebral vascul l ion	?	60		
14	6	61	10	Ci culatory i suffi iency	57	59	60	
15	2							
16	3							
17	5							
18	2				65			
19	p.m.	70	14	Circul ry i suffi iency Uraemi	60	65		Ca myelography
20	5				33	36		A t lal i suffi iency f the leg lography
21	3	68	4	Circul tory i suffi iency	65	66	67	O ati hyperten ion
22	5	70	7	Circulat ry i suffi iency	65	67	68	Progres ive muscular dy rophy
23	13				52			Peri l ion ? l with myelopathy (withou ? l)
24	9	44	12	Cerebral thrombosi	54	63		
	14							
				Pulmonary embolism, U ?al l ?al is. P face ion f small ion	1	63		
					57			

28	7	83	8					
29	4							
30	8							Myelography
31	14							
32	2				55	60		
33		57	7					
34	..	53	7	cal tery insu ciency Uremi	50	85	86	Pancrea i insufficiency Malabsorption with polyneu opathy
35	5				80	61		Chronl nete Iti
36	8	85		cu tery insuff enc	40			
	3							Amyotrophi l teral sclerosi
38								
39								
40								
		83		Acute cha ang tie. rculatory insu f	7			
43	..	83		i cul tery i suf ciency	50		81	Alcoholi polyneuropathy
45	2				88	40		
46					37			Myelography
47	7	89	9	Circulatory insuffi ency	85			Arte ial Insufficiency f the legs. Arte iography Myelography
48	3				83	87		Amytrophi lateral sclerosi
49	3							
50								
51	5							Arte ial insufficiency of the legs. Arte iog aph
52	..	5	8	Circulatory i su fi iency Bronchopneum.				
53		78	13		80			Myelography Exploration of lumbo spinal cord Addison disease?
54	8				7			
55	8							The cutanea tarda type f porphy i
56								Irritable colon
57	4							
58	1							
59	3							
60	3							

Table III Age when the diagnosis of amyloidosis was histopathologically confirmed ante mortem in 49 patients with amyloidosis and polyneuropathy

		Age at diagnosis (years)			
Group	No	Range	Median	Mean	S D
Total	49	34 - 83	61	59 8 ± 1 84	12 9 ± 1 29
Male	33	34 - 83	63 5	61 8 ± 2 36	13 6 ± 1 67
Female	16	35 - 75	59 5	55 5 ± 2 95	11 8 ± 2 08
Familial cases	33	34 - 83	61	60 1 ± 2 29	13 2 ± 1 62
Male	22	34 - 83	66	63 9 ± 2 83	13 3 ± 2 00
Female	11	35 - 63	55	52 5 ± 2,95	9 8 ± 2 08
Probands	21	34 - 83	66	63 9 ± 2 88	13 2 ± 2 03
Secondary cases	12	35 - 68	56	53 5 ± 3 12	10 8 ± 2 20
Sporadic cases	16	38 - 75	58 5	59 1 ± 3 17	12 7 ± 2 24
Male	11	38 - 75	57	57 7 ± 3 73	12 4 ± 2 64
Female	5	39 - 75	64	62 0 ± 6 33	14 2 ± 4 49

Table IV Age at onset of symptoms in patients with amyloidosis and polyneuropathy

		Age at onset of symptoms (years)			
Group	No	Range	Median	Mean	S D
I <u>All patients</u>					
Total	60	29 - 75	53 5	53 0 ± 1 47	11 4 ± 1 04
Male	40	29 - 75	55 5	54 4 ± 1 88	11 9 ± 1 33
Female	20	33 - 70	51 5	50 1 ± 2.17	9 7 ± 1 53
Familial ases	42	29 - 75	54	52.6 ± 1 80	11 7 ± 1 27
Male	27	29 - 75	56	55 0 ± 2.46	12.8 ± 1 75
Femal	15	33 - 63	50	48 3 ± 2 14	8 3 ± 1 50
Non-familial cas e	18	35 - 72	54	53 9 ± 2 59	11 0 ± 1 83
II Patient diagnosed <u>ante mortem</u>					
Total	49	29 - 75	55	53 7 ± 1 05	7 4 ± 0 74
Male	33	29 - 75	56	55 9 ± 2.14	12.2 ± 1 50
Female	16	33 - 67	51 5	49 1 ± 2.52	10 1 ± 1 78
Familial c ses	33	29 - 75	54	53 4 ± 2 14	12 3 ± 1 51
Male	22	29 - 75	56	56 9 ± 2.75	12.9 ± 1 93
Female	11	33 - 56	47	46 3 ± 2.34	7 8 ± 1 66
Probands	21	29 - 75	56	56 3 ± 2 83	13 0 ± 2 00
Secondary cases	12	33 - 63	51	48 3 ± 2 69	9 7 ± 1 97
Non-familial cases	16	35 - 72	55	54 5 ± 2 87	11 5 ± 2 03
Male	11	36 - 72	53	53 8 ± 3 57	11 2 ± 2 38
Female	5	35 - 70	55	56 0 ± 5 98	13 4 ± 4 24
P tients with diarrhoea[a]	16	?9 - 69	50	49 2 ± 2 85	11 4 ± 2.01
Patie t without diarrhoea[a]	33	33 - 75	53	55 6 ± 1 37	7 9 ± 0 97

[a] See Table I col mn 11

Table V Age at death of 27 patients with amyloidosis and polyneuropathy

Group	No	Age at death (years) Range	Median	Mean	S D
Total	27	48 – 85	66	65 6 ± 1 90	9 9 ± 1 34
Male	20	48 – 85	66 5	66 4 ± 2 41	10 8 ± 1 70
Female	7	53 – 71	66	63 3 ± 2 45	6 5 ± 1 74
Patients with diarrhoea[a]	12	48 – 71	61	61 1 ± 2 13	7 4 ± 1 51
Patients without diarrhoea[a]	15	54 – 85	68	69 2 ± 2 66	10 3 ± 1 87

[a]See Table I column 11

Table VI Duration from onset of symptoms to death in 27 patients with amyloidosis and polyneuropathy

Group	No	Duration from onset to death (years) Range	Median	Mean	S D
Total	27	4 – 31	9	10 7 ± 1 17	6 1 ± 0 82
Male	20	4 – 31	9	11 3 ± 1 54	6 9 ± 1 09
Female	7	5 – 12	8	8 7 ± 1 01	2 7 ± 0 72
Patients with diarrhoea[a]	12	4 – 9	7 5	7 3 ± 0 57	2.0 ± 0 40
Patients without diarrhoea[a]	15	5 – 31	12	13 4 ± 1 80	7 0 ± 1 27

[a]See Table I column 11

Table VII Subjects belonging to family 1 and 2 in whom polyneuropathy was obvious but in whom no amyloid deposits were found at histopathological examination

Ge etical meral	Sex	Age	Clinical grading of neuropathy[a] Arms	Legs	EMG confirming europathy	No amyloid detected in tissues examined: Biopsy	Autopsy
1:IX:26	M	60	+ +	+ + +	Yes	Nerve x2[b] skin rectum x2 muscle	Heart lung liver muscle kidney spinal cord
1:IX:27	M	57	+	+ +	Yes	Nerve x2 skin x2 rectum muscle x2 liver esophagus	
1:IX:32	M	66	+ +	+ + +	Yes	Nerve skin rectum x2 muscle	
1:X:4	M	27	+	+	Yes	Nerve skin rectum x2	
1:X:5	M	54	+ +	+ + +	Yes	Skin rectum	
1:X:9	F	31	+	+	Yes	Skin muscle	
2:IV:38	M	70	+	+ +	Not examined	Skin	
2:IV:47	M	78	+	+ +	Not examined	Skin rectum	

[a] + slight; + + moderate; + + + = marked neuropathy

[b] 2 biopsy and examination at two different occasions

Table VIII Results of histopathological examination of biopsy specimens concerning amyloid deposits correlated to clinical grading of polyneuropathy

Results of histopathological examination[a]	Group	Number of patients	Clinical grading of the polyneuropathy in the lower limbs[b] +	+ +	+ + +
Amyloidosis confirmed	Total	49	8	15	26
	Family 1	6		1	5
	Family 2	8	3	1	4
No amyloid detected	Family 1	6	2[c]	1[c]	3[c]
	Family 2	16	14[d]	2[c]	

[a]Examination in polarized light after staining with alkaline Congo red

[b]+ = slight; + + = moderate; + + + = marked polyneuropathy [c]Details are given in Table VII [d]In most of these cases the signs and/or symptoms of polyneuropathy were slight Only skin biopsy was performed

Table IX Sibships with at least one affected member

Affected Male	Female	Total	Normal sibs	Total sibs	Number of sibships
48	28	76	160	236	41

Table X Survey of sibships according to number of affected individuals and size of sibships

		s = 1	2	3	4	5	6	7	8	9	10	11	S
	1	1	3	5	1	2	3	1	3	1			20
	2			2	1	2		2	2	1	1	2	13
r =	3				3			1	1				5
	4						1						1
	5									1			1
	6									1			1
	S_r sr	1	3	7	5	4	4	4	6	4	1	2	41
r_{min} 1	S_r rn_s	1	3	9	12	6	7	8	10	14	2	4	76
	S_r sn_{sr}	1	6	21	20	20	24	28	48	36	10	22	236

s = size of sibships r = number of affe ted sib n_{sr} = sibships of size s comprising r off ted sibs in sibships involving at least one (r_{mi} =1) affected sibs

LEGENDS Pedigrees 1 -15

☐ Male ☐ Both sexes

○ Female ④ Four children

Died

Died before 25 years of age
25

Polyneuropathy diagnosed at examination

Signs and symptoms indicative of incipient polyneuropathy

Reliably reported by relatives as polyneuropathy

Amyloidosis proved histopathologically

Biopsy negative for amyloidosis

• Examination performed by the author

Consanguinity

P obend

☐ Numeral below symbol denotes number of
30 the individual in the generation

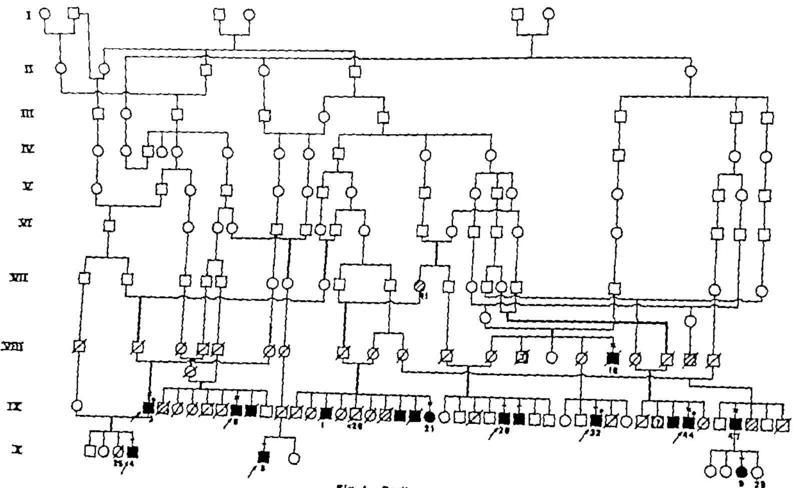


Fig 1 Pedigree 1

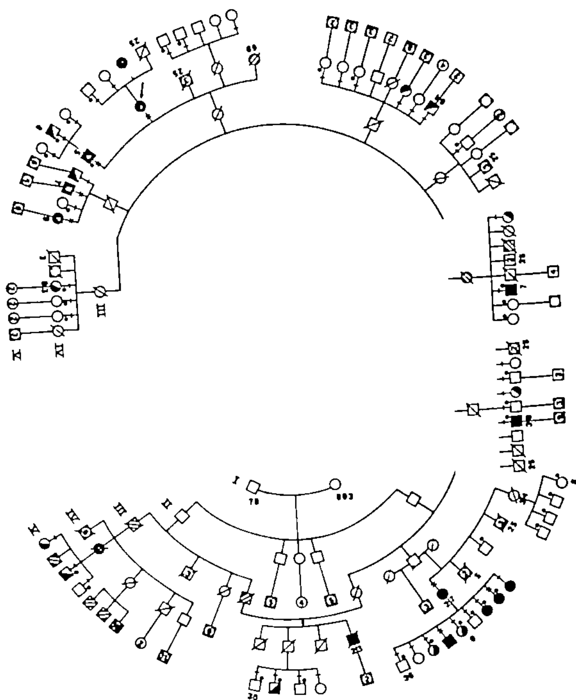


Fig 2 P dig 2

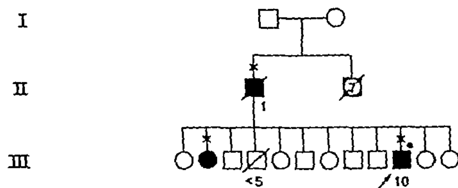


Fig 3 Pedig ee 3

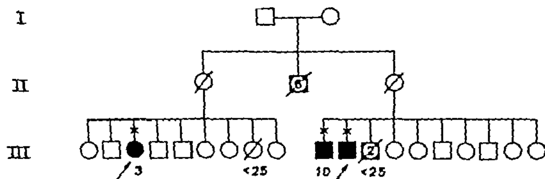


Fig 4 Pedig ee 4

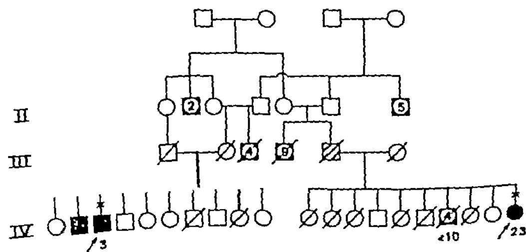


Fig 5 Pedig ee 5

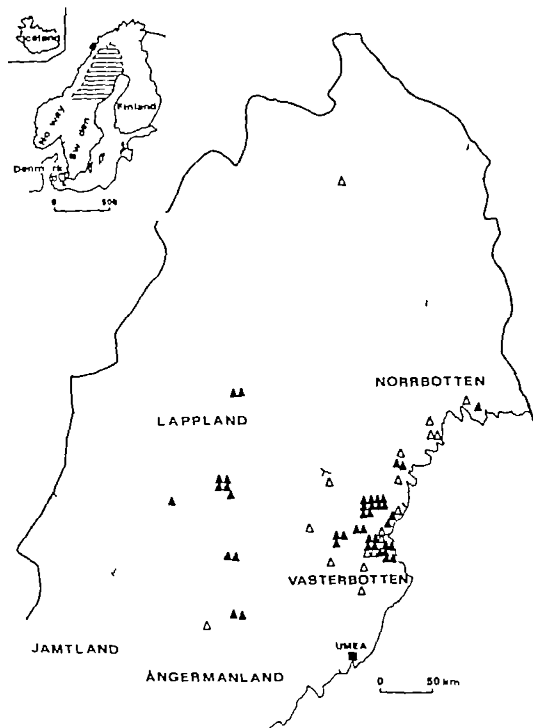


Figure 16 Map of Northern Sweden with birthplaces of 60 patients with amyloidosis and polyneuropathy. Familial cases are designated by solid and sporodic cases by non-filled symbols. A map of the Nordic Countries is inserted with the relevant part of Northern Sweden indicated by lines

Printed in Sweden, Ågrens Boktryckeri AB, [illegible] [illegible]